THE URINE
THE COMMON POISONS
and
THE MILK

Prof. J. W. Holland

THE URINE,

THE COMMON POISONS,

AND

THE MILK.

MEMORANDA,

CHEMICAL AND MICROSCOPICAL,

FOR

LABORATORY USE.

BY

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PREFACE.

THIS book is intended to be used as a syllabus for the laboratory. The text is made brief and to the point, so as to make a pocket volume, handy for reference. Pages are left blank for calculations, memoranda, or more extended notes to be made by the student. For the convenience of those whose course of study is very short, the most important matter is printed in the larger type. Provision is made for more thorough study by the explanations and quantitative processes given in the small print.

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COMPOSITION

OF

HEALTHY URINE.

Healthy urine is a clear yellow fluid, slightly acid in reaction, and holding in solution variable amounts of substances organic and inorganic.

Average Composition of Normal Urine.	Percentage Composition.	Grains per Diem.
Water,	96.0	50 fl. ozs.
Solids, as tabulated below,	4.0	1000 grs.
Urea,	2.000	500.0
Uric Acid,	.040	10.
Hippuric Acid,	.075	15.
Creatinine,	.075	15.
Pigment, Mucus, Xanthine, other extractives, etc.,	1.000	170.
Chlorides of Potassium and Sodium,	1.000	170.
Sulphates of Potassium and Calcium,	.110	40.
Phosphates of Potassium and Sodium,	.120	45.
Phosphates of Magnesium and Calcium,	.080	30.

Beside these there have been found traces of Indican, Diastase, Oxalic, Lactic and Carbolic acids, Unoxidized Sulphur and Phosphorus.

EXAMINATION

OF

MORBID URINE.

THE SAMPLE.

In casual examination for the presence of abnormal constituents, the sample of urine may be voided at any hour of the day. If a single discharge is used, that made on rising is to be preferred.

For accurate results, it is necessary to take a portion of the *total quantity* passed in the twenty-four hours. To obtain this, the patient is instructed to throw away the urine voided at an hour noted, say 9 A. M. Every evacuation of the bladder after this hour must be made into one vessel, such as a large "specie jar," which should be well cleaned daily and kept in a cool place.

At the same hour of the following day the urine is passed into this collecting jar, and the amount measured and noted. (The mean daily discharge is 1250 c.c., or f℥ 40–50, or 2½ pints.) About a half pint of this should be put into a clean glass bottle, with a fresh stopper, and examined within twelve hours at furthest. As a rule, it is best to examine within six hours after emission.

Urine kept in a warm place soon passes into putrefaction, and then is not suited for analysis. Putrefactive changes can be prevented by adding to half a pint of urine as much salicylic acid as can be taken on the point of a penknife, about three grains. Without putrefaction, some organized deposits, as blood, tube casts and renal epithelium, are altered, if not broken up, by urines of low density. If it is desired to preserve a specimen of these organized deposits the clear urine should be decanted, leaving the

sediment, which can be washed free of stale urine by pouring upon it a filtered strong solution of potassium acetate with a small quantity of salicylic acid dissolved in it. After standing long enough to allow the sediment to fall, the clear fluid should again be decanted and a fresh portion of the salicylated acetate of potassium solution be poured on.

By repeating this operation several times all putrescible matter can be removed and the cells, etc , be left in a permanent medium. In examining urine under the microscope for casts, epithelial cells and other organic bodies, difficulty is sometimes caused by suspended urates; to obviate this difficulty and to preserve the specimen, Wendringer advises that the urine should be mixed with a nearly saturated solution of borax and boracic acid. This dissolves the urates and prevents fermentation. The solution is prepared by mixing twelve parts of powdered borax in one hundred parts of hot water, and then adding a similar quantity of boracic acid, stirring the mixture well. It is filtered while hot. The urine to be examined is put into a conical glass, and from a fifth to a third of its bulk of the boracic solution added to and agitated with it. The urine will be found to have become clear in a short time—*i. e.*, if there is no cloudiness due to bacteria; and if a third of its bulk is added, no fermentation or putrefactive processes take place, even if the glass is left uncovered in warm places. Albumin, too, if it exists, is not coagulated. The organic elements —as epithelial cells, casts, blood corpuscles, etc —subside quickly, without undergoing change.

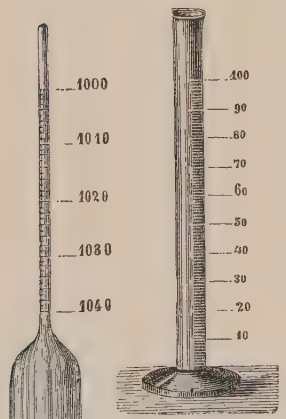
1. *Experiment.*—Having noted the characters of a sample of fresh urine, let it stand aside in a warm place for a few days, and then mark the change in odor, specific gravity, reaction and sediment. A healthy urine may first become acid, depositing urates and uric acid, but all samples ultimately become ammoniacal, putrid, and throw down a sediment of phosphates.

COLOR.

In recording color, it is well to take as a standard Vogel's scale, found in large treatises. The urine is *pale yellow* after free potations, after hysterical or epileptic attacks and in diabetes; *orange red* by the presence of santonin when alkaline; *reddish* with scant potations, after heavy

meals, much exercise, or copious sweating, after hemorrhage into any part of the urinary tract and during fever; after the administration of logwood; *brownish* in melanosis and after the administration of senna, gallic acid, tannic acid or carbolic acid. The presence of biliary coloring matter imparts an intense yellow or brown or olive-green hue; it is characteristic of jaundice. Blood gives a scarlet or a smoky-brown tint. The presence of Indican or indigo-forming substance is shown by adding to normal urine $\frac{1}{10}$ its volume of hydrochloric or nitric acid. By boiling, or even by standing awhile, the color deepens or changes to pink, from the formation of Indigo-red.

Fig. 1.



Graduated Cylinder for measuring the amount of Urine.

Urinometer.

GMELIN'S TEST FOR BILE PIGMENTS.

2. Put some *yellow nitric acid* (*i. e.*, containing lower oxides of nitrogen) into a test tube and overlay it with biliary urine. At the line of junction there will rise, in the following order, bands of green, blue, violet, red and yellow. The green and red will be the most persistent.

The same play of colors can be seen if the test is performed by bringing together on a white plate or piece of white filter paper the urine and the nitric acid. If the amount of bile color is very small, yielding a doubtful result, it can be concentrated by shaking a quantity of urine with chloroform and then separating the chloroform by decanting the urine. If the chloroform is now overlaid with nitric acid the colors will appear at the line of contact.

3. *Oliver's Test for the Biliary Acids.*—The reagent is prepared by dissolving 30 grains of pulverized meat peptone, 4 grains of salicylic acid, and 30 minims of strong acetic acid, in sufficient water to make 8 fluid oz.

Preserved by the salicylic acid the peptone shows no signs of decomposition on keeping. To use the test, mix 1 fluid drachm of the reagent with 20 minims of urine, previously diluted to a standard specific gravity of 1.008. A faint haze is produced after a minute by the trace of biliary salts in normal urine, but if these are in excess a milky cloud promptly appears, which will be more or less distinct, according to the proportion of bile salts present.

Pettenkofer's test is not practicable for urinary analysis.

ODOR.

It is easy to detect a departure from the familiar odor of health. Some articles of food, as asparagus, and some aromatic medicines, impart a change. Saccharine urine has a fruity odor. Putrid urine has the odor of ammonia modified.

SPECIFIC GRAVITY.

4. *Test.*—In taking the specific gravity, if the containing vessel has a level lip at its mouth, the most accurate reading is made by immersing the urinometer while the vessel is half-full of urine, and then carefully filling it to the brim. The exact line of the registry can be taken by sighting horizontally the surface of the urine which rises slightly about the stem of the instrument.

For scientific accuracy, the specific gravity bottle and balance are to be preferred.

Import.—The healthy standard is between 1015 and 1025. If persistent below 1015, Albuminuria or Diabetes Insipidus may be suspected.

If persistent above 1030, Diabetes Mellitus probably exists. Further examination is necessary to make its meaning clear.

SOLID URINE.

5. Roughly estimate the solid urine in parts, per fluidounce, by Bird's formula. The two last figures of the spec. gravity nearly represent the grs. of solids to the

fluidounce. The same figures multiplied by 2 (Trapp's factor) will give, more accurately, parts per 1000. For example, if the spec. gravity is 1025, then there are 25 grs. of solids to the fluidounce of urine, or 50 parts in the thousand. Haeser's factor is 2.33, which in a large number of samples is no more accurate than Trapp's.

The most exact method is to evaporate a given amount to dryness on a water bath and then weigh the residue.

In practice this is found too tedious for clinical use.

REACTION.

6. *Test.*—First put a drop of the sample on a piece of blue litmus paper; if the blue turns to red, then the reaction is *acid;* if the paper is unchanged, then it will be *alkaline* or *neutral.* Now try it with red litmus paper; if it turns blue, the reaction is *alkaline;* if the paper is unchanged, the urine is *neutral.* Violet-colored "neutral litmus" paper alone can be used. It will turn reddish with acids and bluish with alkalies.

The degree of acidity can be determined by the process described, p. 61, using decinormal solution of potassium hydrate (5.6 in 1000), and reporting in terms of oxalic acid. Each c.c. of the titrating solution representing 0.006 gm. oxalic acid in the volume of urine used.

If alkaline, it may be so from salts of potassium and sodium (fixed alkali) or from ammonia (volatile alkali). If due to ammonia, then by exposure the ammonia vaporizes and the red color of the paper is restored; whereas, the blue from fixed alkali remains.

The normal reaction of the total daily discharge is acid. It can be made alkaline by the administration of alkaline medicines.

Urine is made ammoniacal by the decomposition of urea.

$$\underset{\text{Urea.}}{CON_2H_4} + \underset{\text{Water.}}{2H_2O} = \underset{\text{Ammonium Carbonate.}}{(NH_4)_2.CO_3}.$$

CHLORIDES.

The chlorine in the urine is in combination principally with sodium as NaCl. The mean daily discharge is 200 grs., or 12 gms., amounting to more than all the other salts together. The chlorides diminish when exudations are forming, and in Pneumonia may disappear entirely.

7. *Test for Chlorides.*—First, add a few drops of nitric acid to prevent the precipitation of phosphates, then on the addition gradually of solution of silver nitrate a white precipitate of silver chloride forms.

By comparison of this precipitate with that from a sample of normal urine a rough estimate can be made of the relative amount of chlorides.

$$NaCl + AgNO_3 = NaNO_3 + AgCl.$$

8. *Volumetric Determination.*—See p. 61 for rules to be observed. Dilute 10 c.c. of urine with 50 c.c. of water. Put this into a beaker or capsule, with a few drops of neutral potassium chromate. Add gradually from a burette (Fig. 2) the standard solution of silver nitrate until the urine is permanently orange red; then note the c.c. of reagent used, subtracting 1 c.c. for excess of silver solution. The standard solution contained $AgNO_3$ 29.06 gm. per 1000 equal to 10 gms. NaCl, then 1 c.c. = .01 NaCl in the 10 c.c. of urine used, or 0.1 per cent.

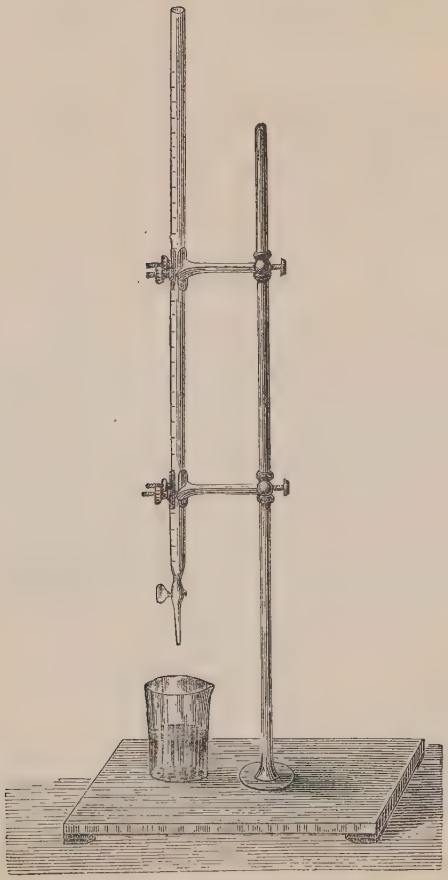
By multiplying the number of c.c. noted by 10, the percentage can be obtained, or if multiplied by 45.57, the number of grains to the fluidounce of urine. Then, if 15 c.c. were used, 14 c.c. would be counted.

1 c.c. = 0.01 NaCl in 10 c.c. of urine.
14 c.c. = 0.14 " " "
= 1.40 " 100 " "

1.40 per cent. multiplied by 4.55 = 6.37 grains to the fluidounce.

This method, which serves well enough for clinical study, cannot be considered scientifically exact when the urine is high-colored, or putrid, or albuminous. To make it satisfactory, the organic compounds may be removed by boiling 10 c.c. of the urine and adding to it, drop by drop, a solution of potassium permanganate, until a faint pink hue is left. The brown precipitate formed is separated by filtration. Before titration the pink tint should be removed by a few drops of oxalic acid, the urine made neutral with calcium carbonate, and filtered.

FIG. 2.



Graduated Burette.

SULPHATES.

The compounds of sulphur are derived from food and the oxidation of proteids of the body. They are discharged daily to the amount of about 30 grs. or 2 gms., mostly with alkaline bases, but partly with organic compounds.

9. *Test.*—Into the test tube containing the urine put a few drops of hydrochloric acid to prevent the formation of barium phosphate. On adding solution of barium chloride, a white precipitate falls, varying in density from a cloudiness to a creamy consistency. Compared with the results obtained from equal amounts of healthy urine acidified, one can judge as to whether the sulphates are greater or less than normal.

10. *Volumetric Estimation.*—Into a beaker on a sand bath put 100 c.c. of urine acidulated with hydrochloric acid, and boil it. From a burette drop in the standard solution of barium chloride as long as the precipitate forms. Set aside for subsidence of the precipitate. Now cautiously run one drop of the barium chloride solution down the side of the beaker to see if there will be further precipitation. Run in the barium chloride until the white cloud no longer increases. To determine if too much of the barium chloride has been used, filter a few drops out of the beaker and test it with a drop of sodium sulphate. A white precipitate indicates excess of the standard solution, and calls for a repetition of the titration.

The standard solution contained 30.5 gm. $BaCl_2$ in 1000 c.c. and 1 c.c.= 0 01 gm. SO_3 in the 100 c. c. of urine used. If 15 c. c. were needed, then there were present 0.15 gm. SO_3 in the hundred.

To get grains in the fluidounce multiply by 4.557.

Import.—As yet no such correspondence has been detected between the amount of sulphates and any pathological process as to make an estimate of value in diagnosis.

PHOSPHATES.

The phosphoric acid of urine is united partly with the earthy bases, as acid calcium and magnesium phosphates, and partly with alkaline bases, as acid sodium phosphate and a trace of acid potassium phosphate. The average daily output is 60 grs. or 4 gms., of which two-thirds are joined to

alkaline bases and one-third to earthy. As the latter are insoluble in alkaline fluids, they are deposited whenever the urine is made alkaline by an internal course of such remedies, or by putrefaction, changing its urea into ammonium carbonate.

$$\underset{\text{Urea.}}{CON_2H_4} + 2H_2O = \underset{\text{Ammonium Carb.}}{(NH_4)_2CO_3}.$$

$$\underset{\text{Magnesium Phosphate.}}{2(MgHPO_4)} + (NH_4)_2CO_3 = H_2CO_3 + \underset{\text{Triple Phosphate.}}{2(MgNH_4PO_4)}.$$

11. *Test.*—To the urine in a test tube add a few drops of potassium hydrate, and boil. The earthy phosphates are thrown out and must be separated by filtration.

12. *Test.*—To the filtrate add one-third its volume of "magnesia mixture." The precipitate formed represents the phosphoric acid once held by alkaline bases, now in the form of ammonio-magnesium phosphate (triple phosphate).

13. *Teissier's Method.*—To get a fair idea of the quantity of phosphoric acid in urine, we can resort to the following easy method: Into a cylindrical vessel, graduated in c.c., measure 50 c.c. of urine. To this add "magnesia mixture" a sufficient quantity, at least 15 c.c. After shaking together set aside for twenty-four hours. As triple phosphate all the phosphoric acid will be thrown down in a compact white sediment, of which 1 c.c. in height represents 0.30 gm. per litre, or 0.03 per cent of phosphoric acid. This is equivalent to 0.60 to 0.70 gm. of phosphates per litre, or 0.06 to 0.07 per cent.; multiplying this percentage by 4.55 will give the amount in grs. to the fluidounce.

FIG. 3.

Deposit in ammoniacal urine (akaline fermentation). (*a*) Acid ammonium urate; (*b*) ammonio-magnesium phosphate; (*c*) bacterium ureæ.

14. *Volumetric Estimation.*—Having measured 50 c.c. of urine, pour it into a capsule resting on wire gauze or a sand bath. Add to this 5 c.c. of a solution of sodium acetate, which has an excess of acetic acid. Heat to boiling, and slowly add from a burette the standard solution of uranium acetate. A precipitate forms, of uranium phosphate, insoluble in the acetic acid. With a glass rod stir the mixture, and at intervals let a few drops fall on a solution of potassium ferrocyanide in a white plate, to see if it will form a red-brown spot, indicating that excess of uranium acetate has been added.

Note the number of c.c. of standard solution used up to that point.

The standard solution of uranium acetate contained 31.1 gm. to 1000 c.c. equal to 5 gm. P_2O_5. As 1000 c.c. = 5 gm. P_2O_5, then 1 c.c. of the test will represent 0.005 gm. P_2O_5 in the 50 c.c. of urine taken. To get per cent., multiply the number of c.c. used by 0.01, which is the equivalent of 0.005×2. The per cent. multiplied by 4.55 will give grains to the fluidounce.

Import.—A knowledge of the relative amount of phosphates in a sample of urine is of no service in diagnosis or treatment.

The clinical significance of the phosphates depends not on the amount, but on their state.

When they are persistently thrown out from solution, there is reasonable ground to fear that in time a gravel or calculus may be formed.

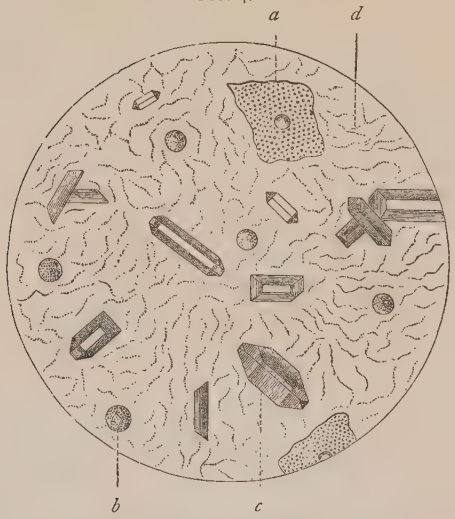
When retained in the bladder and when from cystitis the urine is purulent and rapidly putrescible, passing into ammoniacal fermentation, the alkaline reaction induced by the ammonia causes the precipitation of the earthy phosphates before micturition.

Phosphatic Deposits.—The amorphous phosphate deposit occurs whenever the urine becomes alkaline, is grayish-white and composed of the phosphates of calcium and magnesium. Under the microscope it appears as granules, without color or tendency to aggregate in masses of any particular shape.

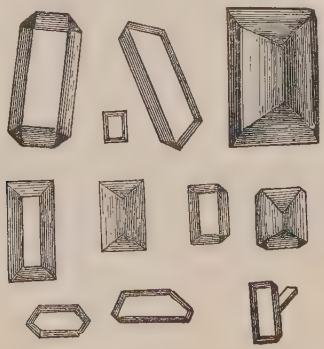
The granules of amorphous *urates* usually are pinkish, arranged in delicate moss-like forms, and disappear on the addition of potassium hydrate.

Under this reagent the phosphates are unchanged, but they clear up when a drop of acetic acid is introduced under the cover glass.

FIG. 4.



Deposit from a case of inflamed bladder (ammoniacal fermentation). (*a*) Detached epithelium; (*b*) pus corpuscles; (*c*) triple phosphate; (*d*) microörganisms.



The more usual forms of triple phosphate × 300.

Triple or Ammonio-magnesic Phosphate.—This is the combination in which the phosphoric acid falls after putrefactive changes. To the naked eye it is a whitish

sediment in a turbid fluid. Sometimes the crystals may be detected as brilliant white points on the sides of the glass, or as a shining film on the surface of the urine.

Under the microscope most of these crystals are prismatic and have some resemblance to a "coffin lid." They are colorless, bright, relatively large, and easily distinguished from the spiny spherules of ammonium urate which often accompany them.

Import.—Their presence denotes ammoniacal decomposition of the urine. If this condition exists at the time of micturition, it is evidence of cystitis or other disease of the genito-urinary tract. If persistent, there is liability to the formation of phosphatic calculus.

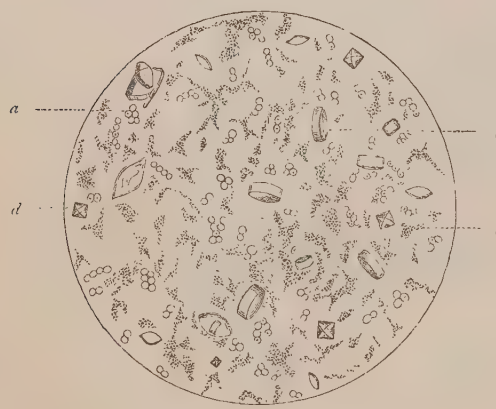
URIC ACID.

$C_5H_4N_4O_3$.

Uric acid, like urea, is nitrogenous. The quantity eliminated daily is 0.7 gm. or 10 grs.

In healthy urine it exists only in combination, as potassium and sodium urates. Acid and dense urine, on standing, will undergo change, becoming more acid and throwing down a deposit of uric acid, calcium oxalate and amorphous sodium urate.

FIG 5.



Deposit in "acid fermentation" of urine. (*a*) Fungus; (*b*) amorphous sodium urate; (*c*) uric acid; (*d*) calcium oxalate.

Free uric acid may exist in the urine at the time it is voided, but in any but the smallest amount must be considered as pathological. It is practically insoluble, requiring 18,000 parts of water to dissolve it. It is freely soluble in the alkalies and solutions of the alkaline carbonates.

15. *Separation of Uric Acid.*—To 100 c.c. of urine add 10 c.c. of hydrochloric acid; let it stand for 48 hours, when the uric acid will be settled as fine crystals, looking like grains of Cayenne pepper.

16. *Estimation.*—Having obtained the crystals as above, decant the urine and stir the sediment with 30 c.c. of water, using for this purpose a glass rod with a piece of rubber tubing at the end. Throw the suspended crystals on a weighed filter, dry over a water bath and weigh.

This method, though simple, is approximative only.

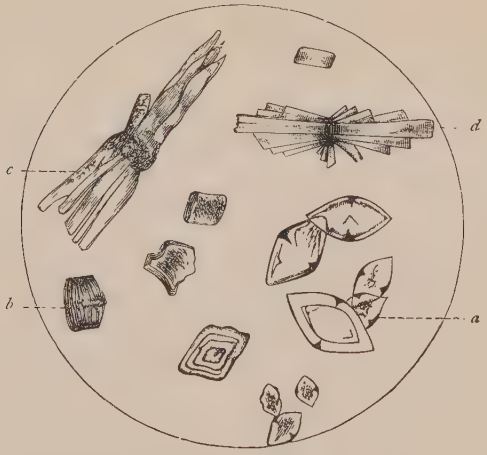
17. *Haycraft's method* is to be preferred where scientific precision is desired and a well furnished laboratory is at command.

The method in detail has been given as follows:—

The following solutions must be prepared: 1. Dissolve 5 grammes of nitrate of silver in 100 c.c. of distilled water, and add ammonia until the precipitate first formed redissolves. 2. Dilute strong nitric acid with about two volumes of distilled water; boil, to destroy the lower oxides of nitrogen, and preserve in the dark. 3. Dissolve about 8 grammes of ammonic sulphocyanide crystals in a litre of water, and adjust to decinormal argentic nitrate solution, by diluting till one volume is exactly equal to a volume of the latter. Dilute the solution thus prepared with nine volumes of distilled water, and label "Centinormal ammonio-thiocyanate solution." 4. A saturated solution of ferric alum. 5. Strong solution of ammonia (sp. gr. 0.880). The uric acid estimation is conducted as follows: Place 25 per cent. of urine in a beaker with 1 gramme of sodic bicarbonate. Add 2 or 3 c.c. of strong ammonia, and then 1 or 2 c c. of the ammoniated silver solution. If, on allowing the precipitate caused by the latter reagent to subside, a further precipitate is produced by the addition of more solution, the urine contains an iodide, and silver solution must be added till there is an excess. The gelatinous urate must now be collected, the following special procedure being necessary: Prepare an asbestos filter by filling a 4-oz. glass funnel to about one-third with broken glass, and covering this with a bed of asbestos to about a quarter of an inch deep. This is best managed by shaking the latter in a flask with water until the fibres are thoroughly separated, and then pouring the emulsion so made in separate portions on to the broken glass. On account of the nature of the precipitate and of the filter it is necessary to use a Sprengel pump or aspirator in order to suck the liquid through. Having collected the precipitate of silver urate on the prepared filter, wash it repeatedly with distilled water, until

the washings cease to become opalescent with a soluble chloride. Now dissolve the pure urate by washing it through the filter with a few cubic centimetres of the special nitric acid. Place in a beaker, and estimate the silver by Volhard's method, which can be employed in the presence of nitric acid. The process is carried out thus: Add to the liquid in the beaker a few drops of the ferric-alum solution to act as an indicator, and from a burette carefully drop in centinormal ammonic thiocyanate until a permanent red coloration of ferric-thiocyanate barely appears. The number of cubic centimeters used of the thiocyanate solution multiplied by 0.00168 gives the amount of uric acid in the 25 c.c. One milligramme may be

FIG. 6.



Uric Acid. (*a*) Rhombic tables (whetstone form); (*b*) barrel form; (*c*) sheaves; (*d*) rosettes of whetstone crystals.

added to compensate for loss, and the whole multiplied by four gives the percentage of uric acid in the urine. The whole process depends on the fact that argentic urate fails to dissolve in ammonia, but is soluble in nitric acid, and is thus easily obtained in the pure state. By determining the amount of combined silver, the percentage of uric acid can readily be calculated. The addition of sodic bicarbonate prevents the otherwise inevitable reduction of the silver salt.

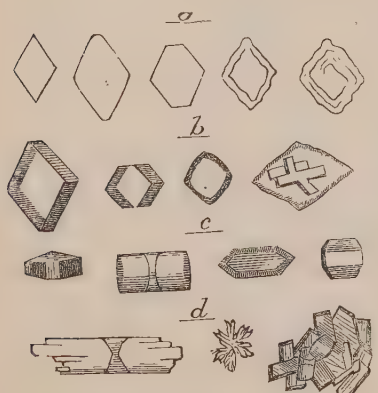
18. *Murexide Test.*—Put any substance supposed to be uric acid or urates into a watch crystal or a porcelain

dish. Dissolve in strong, cold nitric acid, and at gentle heat evaporate to dryness. Touch the yellow or red residue with a drop of ammonia water, or simply expose it to the vapor of ammonia. A bright violet blue or purple red (murexide) indicates uric acid.

Deposit of Uric Acid.—While a faint trace of free uric acid may be found in healthy urine, if present in noticeable amount, it is a pathological change. Once deposited, it remains undissolved in acid urine. It is colored by urinary pigment and easily made out by the microscope.

To the naked eye it appears in minute reddish specks. Under the *microscope* these specks are found to be modifications of rhombic crystals. The simpler lozenge forms have a resemblance to a *whetstone*. Some are like a drum, others are like rosettes, others, again, sheaf like bundles.

FIG. 7.



Uric Acid. (*a*) Rhomboidal, truncated, hexahedral, and laminated crystals; (*b*) rhombic prism, horizontally truncated angles of the rhombic prism, imperfect rhombic prisms; (*c*) prism with a hexahedral basic surface, barrel-shaped figure, prism with a hexahedral basal surface; (*d*) cylindrical figure, prism with a superimposed group of crystals.

Import.—Beside its connection with the pathology of gout, uric acid is important as a deposit.

If it is deposited soon after micturition, it is an indication of a tendency to form gravel.

URATES.

It has been said above that uric acid in health exists as neutral urates in combination with sodium principally, but with potassium partly. These urates, in ordinary amount, are soluble in normal urine at common temperatures.

If healthy urine is kept in a cold place, it loses its solvent powers, and the mixed urates are precipitated as a loose pinkish powder at the bottom of the glass and as a film on the surface.

The same thing happens at higher temperatures if the amount of urates is in excess of health and the reaction highly acid. This deposit, often referred to as sodium urate, the brick-dust or lateritious sediment, can be distinguished by its prompt solution on heating the urine. It dissolves readily in potassium hydrate.

19. *Test.*—Having obtained a specimen of urine that is turbid, fill with it a test tube until it is half full. Gently heat the upper half. It will clear up if the turbidity is due to urates.

20. *Test.*—To another portion of the same add potassium hydrate. The sediment of urates will dissolve.

Microscopically (Fig. 5-*b*), the brick-dust deposit is composed of granules usually disposed in moss-like groups. Sometimes these granules have spiny radiations.

The ammonium urate (Fig. 3-*a*) occurs in opaque, brownish spherules with or without spines, and in company with crystals of triple phosphate.

Import.—This deposit is not so important as it is commonly considered, occurring often from such physiological conditions as unusual exercise or sweating. Pathologically, it is associated with fever and chronic hectic diseases.

HIPPURIC ACID,

$C_9H_9NO_3$,

combined with alkaline bases, is a normal constituent. The average quantity eliminated daily is 15 grains. This amount is increased by excess of vegetables in the diet or by large doses of Benzoic acid. If the urine be at the same time diminished in volume the Hippuric acid may be deposited spontaneously. This is, however, an extremely rare occurrence, owing to the solubility of the acid and its salts.

It occurs in Rhombic prisms (Fig. 8), resembling somewhat the coffin-lid crystals of Triple Phosphate. It is to be recognized by the fact that it is precipitated from acid urine only, and does not dissolve when a drop of acetic acid is allowed to flow under the cover glass. The phosphates, on the other hand, are deposited in alkaline urine and clear up in the presence of acetic acid.

CREATININE,

$C_4H_7N_3O$,

is a constituent of normal urine to the amount of about 15 grains daily. It is derived from the Creatin of muscle, is a strong base, alkaline in reaction, and owing to its free solubility in water is very rarely spontaneously deposited from the urine. It forms insoluble compounds with mercuric chloride, with silver nitrate and with zinc chloride. The latter compound has a characteristic crystalline form, by means of which the presence of Creati-

FIG. 8.

Hippuric Acid.

nine may be determined. Under the microscope it is seen as needles arranged in rosettes or balls. It has the power of reducing both cupric oxide and picric acid, and affects to a slight degree the accuracy of estimates of sugar made by reduction tests.

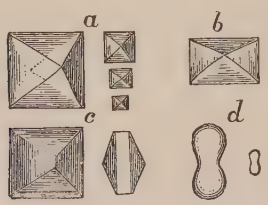
CALCIUM OXALATE

is present in extremely small amount in normal urine. As it takes 500,000 parts of water to dissolve it, the slightest increase over a minute quantity causes it to appear as a deposit.

To the naked eye it appears as a scanty, grayish, mucus-like cloud in the middle or at the bottom of the glass.

Microscopically, it is recognized by one of two forms: the octahedra, usually appearing like bright, square "envelopes," and the dumb bells or hour glasses.

FIG 9.



Oxalate of Lime. (*a*) Octahedra; (*b*) basal plane of an octahedron forming a rectangle; (*c*) compound forms; (*d*) dumb bells.

Perfect dumb-bell crystals of oxalate of lime.

Import.—The deposit sometimes follows the eating of rhubarb or sub-acid fruits containing oxalates. Usually it signifies imperfect oxidation of waste products or retarded metabolism.

If persistent, it may, by accretion form a mulberry calculus.

CYSTIN.

$C_6H_{12}N_2S_2O_4$.

This substance, in an excessively small amount, may be a normal constituent of the urine. It is nearly insoluble in water, and in the very rare cases where it is present in large amount it is in the form of a deposit.

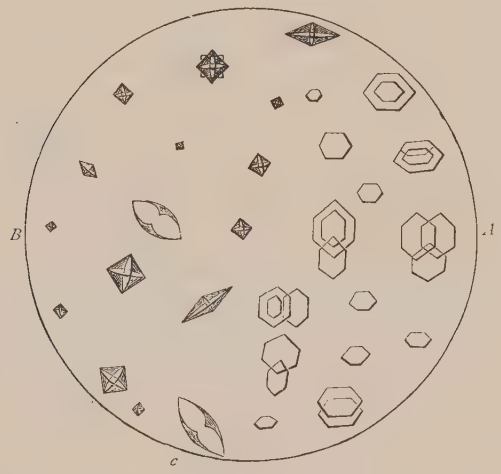
To the naked eye the deposit is usually abundant and light, resembling urates. It is not dissolved by heat or vegetable acids, but readily by ammonia. The ammonia solution exposed on a glass slide deposits crystals which under the *microscope* are seen to be composed of hexagonal tablets. Iodoform escaping into the urine from surgical dressings has been mistaken for it.

21. *Test.*—When it decomposes, hydrogen sulphide is formed. If boiled with a solution of lead oxide in

sodium hydrate, the black lead sulphide forms and is precipitated.

Import.—The conditions of its formation are obscure. It occurs very rarely as a calculus.

FIG. 10.



A, Crystals of cystin; *B*, oxalate of lime; (*c*) hour-glass forms of *B*.

LEUCIN AND TYROSIN.

These substances are formed in the intestines by the same processes of digestion, and in acute yellow atrophy of the liver, typhoid fever and phosphorus poisoning, appear together in the urine.

When present, leucin will appear as a residuum left with tyrosin when the urine is allowed to evaporate on the glass slide. It occurs either as greenish yellow globes with concentric markings or radiating spines.

If a small quantity is put in a watch crystal and saturated with nitric acid, by cautious evaporation to dryness a residue will be left which turns yellow or brown on the addition of solution of sodium hydrate.

Tyrosin may be spontaneously deposited as greenish-yellow crystals composed of bundles of acicular needles arranged in various radiating forms.

Tyrosin turns red when boiled with Millon's reagent (mercurous nitrate); but yields a violet color when first carefully warmed with sulphuric acid and treated with a drop of solution of ferric chloride.

FIG. 11.

(*a a*) Leucin balls; (*b b*) tyrosin sheaves; (*c*) double balls of ammonium urate.

UREA.

$CO(NH_2)_2$ Carbamide.

Urea is the chief compound of nitrogen by which that element is eliminated after having served in the body as a constituent of the proteid principles. The average daily excretion of urea is 40 gms., or 500 grains.

It is neutral, odorless, has a bitter taste, does not affect litmus and plays the part of either acid or basic radical.

It is easily soluble in water and alcohol, but insoluble in ether. It can be prepared by synthesis, and is isomeric with ammonium cyanate.

When crystallized slowly, it forms four-sided prisms with beveled ends.

22. *Test.*—Take a specimen of urine or any fluid suspected to contain urea, concentrate it to a syrupy

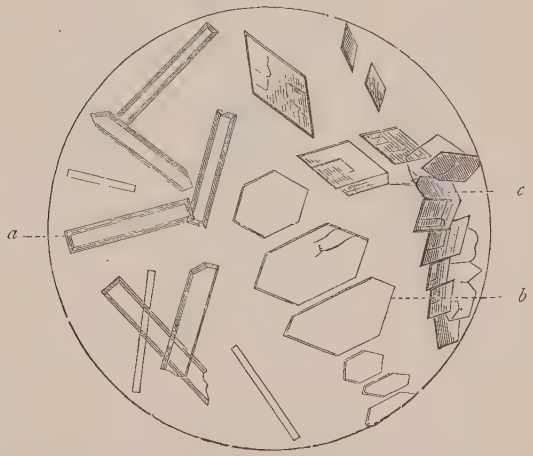
consistence by evaporation on a water bath, add nitric acid. The nitrate of urea will form a crystalline precipitate of rhombic plates (Fig. 12).

Estimation.—Several methods of *quantitative analysis* depend on the power of sodium hypobromite or hypochlorite to decompose urea.

$$N_2H_4CO + 3NaBrO = 3NaBr + CO_2 + 2H_2O + 2N.$$

The amount of urea involved will be indicated either by the loss of specific gravity of the urine or by the volume of nitrogen generated.

FIG. 12.



(*a*) Urea; (*b*) hexagonal plates; and (*c*) smaller scales, or rhombic plates of urea nitrate.

23. *Fowler's Method.*—First, with a urinometer having wide spaces and known to be accurate, note the spec. gravity of the urine, and of the liq. sodæ chloratæ about to be used. In practice the strength of liq. sodæ chloratæ is found to be uncertain. On this account it is best to fortify it by adding a few drops of free bromine. Multiply the S. G. of the mixture by 7 and add the product to the S. G. of urine. Divide the sum by 8 to get the mean S. G.

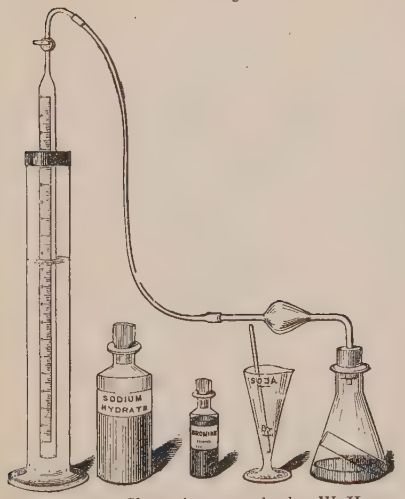
Then to urine f ℥j add liq. sodæ chloratæ f ℥vij. After effervescence ceases, say in two or three hours, take the specific gravity of the mixture.

Deduct this last figure from the mean S. G. obtained in the first place, multiply the remainder by 0.77, and you get the per cent. of urea. To get grains per fluidounce multiply per cent. by 4.55.

Example:—

S. G. of urine = 1020 × 1 volume = 1020
" " liq. sod. chl. = 1025 × 7 " = 7175
1020 + 7175 = 8195
8195 ÷ 8 = 1024 the mean S. G.
1024—1022 (S. G. after mixing) = 2
2 × 0.77 = 1.54 % of urea.

FIG. 13.



Ureameter of Charteris, as made by W. Hume, of Edinburgh.

24. *Russell & West's Method.*—This method measures the nitrogen evolved by sodium hypobromite and uses apparatus which gives N in c. c., each of which represents 0.0027 gm. of urea, or apparatus graduated so as to give percentage (on the principle that 5 c.c. of a 2 % urea solution will evolve 37.1 c.c. of N).

An apparatus often used is seen in Fig. 13.

Solutions of sodium hypochlorite or hypobromite decompose so readily that it is best to use them freshly prepared.

Knop's Solution.—Dissolve 100 gms. sodium hydrate in 250 c.c. of water and add 25 c.c. of bromine.

So important is it that the solution should be fresh, that if any great interval is to occur between successive determinations, it is best to have the fluid in two parts, to be mixed as required. In a separate bottle keep the sodium hydrate solution (1 to 25). To make Knop's fluid add 15 or 20 c.c. of this to 1 c.c. of bromine.

Method.—Fill the glass cylinder (Fig. 13) with water and immerse in it the graduated tube until the zero is at the surface. Into the flask measure 15 c.c. of hypobromite solution. A short test tube containing 5 c.c. of

urine is put in the flask by forceps, so as not to spill. Having closed the flask tightly with a rubber stopper carrying a connection with the graduated tube, tip it gradually, so as to pour out the urine into the hypobromite. Of the gas evolved CO_2 is retained by the soda, while the N passes up the graduated tube. In ten minutes, having raised the collecting tube to the point where its contents are on a level with that of the cylinder, read off the c.c. of nitrogen. Each c.c. = 0.0027 gm. of urea in 5 c.c. of urine used. If it reads 8 c.c. of N then there were $8 \times 0.0027 = 0.0216$ gm. urea in 5 c.c. of urine. This multiplied by 20 to get percentage = 0.432 per cent. Percentage multiplied by 4.55 gives grains to the fluidounce.

Correction for Pressure to 760 mm. and Temperature to 0° C.—For perfect accuracy a correction must be made according to the following formula:—

$$V' = \frac{V\,(b - w)}{760\,(1 + 0.00366\,T)}$$ in which

V′ = volume required; v = volume observed.
b = barometer in m.m.: w = tension of aqueous vapor.
T = observed temperature Centigrade.

25. *Lyon's Apparatus.* * (*Fig. 14.*)—Into the bottle measure 40 c.c. of strong liq. sodæ chloratæ (or, better still, 20 c.c. of fresh Knop's hypobromite solution. As the liq. sodæ chloratæ is often not so strong as is needed, if used at all it is best to fortify it with bromine f℥ss or f℥j of a strong solution of potass. bromide which yields bromine). Fill the little test tube with urine to the 4 c.c. mark, and carefully lower it into the bottle so as not to spill. Hook the bent overflow tube on a bottle or tumbler, and having removed the little rubber cap, fill with water the graduated jar to slightly above zero. Put on the cap far enough to depress the surface of the water exactly to zero. Make all joints tight, and tip the bottle so that the urine slowly flows out, and shake it occasionally. In half an hour lower the end of the overflow tube to the level of the water in the cylinder, and read off the percentage of urea. This multiplied by 4.55 will give grains per fluidounce.

26. *Correction for Pressure and Temperature.*—The graduation of Lyon's instrument is based on the assumption that the temperature is 70° F. (21° C.) and the barometer 30 inches (760 mm.). Any variation from these standards will alter the volume of gas measured.

Barometric changes have so little effect that ordinarily this correction need not be made. *Temperature* variations can be controlled by taking the

* Made by Parke, Davis & Co., Detroit, Michigan.

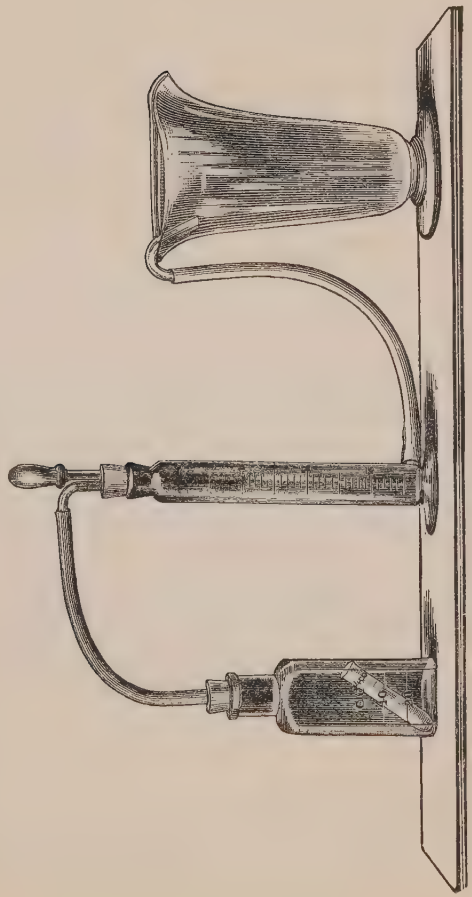


FIG. 14.

observation when the thermometer is near 70° F. Within the usual range indoors the effect on the volume of gas may, for clinical purposes, be ignored. Greater accuracy can be obtained by the following rule: If the temperature is above 70° F., then subtract from the observed volume a correction obtained by multiplying the number of degrees above 70° by 0.0028, and this product by the observed volume. To correct for barometric pressure, subtract from the above volume the amount obtained by multiplying the variation from 30 inches by 0.034, and this product by the observed volume.

27. *Liebig's Volumetric method* depends on the formation of an insoluble compound of urea and mercuric oxide. It is less easy than the hypobromite method, but in the absence of the special nitrogen apparatus it may be resorted to with good results. It requires several solutions. 1st, the *baryta* solution, containing one volume of cold saturated solution of barium nitrate with two volumes of cold saturated solution of barium hydrate. 2d, a saturated solution of sodium carbonate. 3d, a *standard solution of mercuric nitrate*, which is best made by an expert chemist. It can be bought of leading druggists.

Method.—Mix 20 c.c. of baryta solution with 40 c.c. of urine, to precipitate phosphates, sulphates and carbonates. Filter clear and put 15 c.c. of the filtrate into a beaker. Drop from a burette the standard mercuric nitrate solution, testing for excess of reagents by removing a drop at intervals and touching with it the *sodium carbonate* solution on a white plate. When a yellow spot appears the end point has been reached.

Deduct 2 (for effect of chlorides) from the number of c.c. used and multiply by 0.010. This gives the urea in grammes contained in the 10 c.c. of urine operated upon. Again multiplied by 10 gives percentage.

Import.—As urea is highly soluble, it is never spontaneously deposited. It varies in amount with different diseased conditions, *e.g.*, in febrile and inflammatory affections it is increased in the forming stage and diminished in that of defervescence. In diabetes mellitus and simplex it is excessive in the urine, while in acute yellow atrophy of the liver it may be absent entirely.

In acute and chronic Bright's disease there may be a decided falling off from the healthy proportion, causing a lower specific gravity. In such cases there is more or less danger of uræmia. By its ready conversion into irritating ammonium carbonate it has pathological significance in all cases of retention of urine.

SUGAR.

While it is asserted by some that a minute trace of sugar can be detected in healthy urine by the use of tests of great delicacy, the weight of authority at present inclines to the opinion that *normal urine contains no sugar*. It is deemed probable that the reducing power possessed by normal urine (equal to that of a 0.1 per cent. solution of glucose) is due partly to uric acid and partly to creatinine. In diabetes mellitus this power of reducing metallic salts and other compounds is enormously increased, is easily appreciated by ordinary tests and is undoubtedly due to grape sugar, which may be present in the great amount of fifty grains to the fluidounce or 10 per cent.

In the application of any test but that of picric acid, it is necessary to make sure that the urine is *free from albumin*. If any is present, boil the sample with a drop of acetic acid, and filter.

Reduction Tests.—The best tests for glucose are such as Böttger's and Fehling's, which make use of its power to reduce metallic salts to lower oxides or the metallic state when boiled with them in alkaline solution.

28. *Böttger's Test.*—(First. Test for albumin; if present, separate it by acidulating the urine slightly, boiling and filtering). Put into a test tube equal volumes of suspected urine and potassium hydrate, with a pinch of bismuth subnitrate. On boiling for a few minutes, if sugar is present, the white powder turns gray, brown or black, from reduction to metallic bismuth.

Fallacy: Albumin and sulphur compounds in the urine may cause a black precipitate. To make sure, repeat the test, using litharge instead of bismuth. If this blackens, then sulphur compounds have affected the bismuth also, and some other test must be used.

Nylander performs this test with a single alkaline bismuth oxide solution, made as follows:—

℞.	Bismuth subnitr.,	2 gms.	
	Rochelle salts,	4 "	
	Caustic soda (of 8 per cent.),	100 "	Mix.

To 10 c.c. of urine add 1 c.c. of test solution and boil. A brown or black color denotes sugar.

TESTS BY COPPER SULPHATE.

The reducing action of glucose on copper sulphate is subject to conditions which will be understood by performing the following experiments:—

29. In a test tube add to a solution of copper sulphate an equal amount of potassium hydrate; a pale-blue precipitate of cupric hydrate, Cu2HO, forms, insoluble in excess. On boiling, the blue hydrate changes to black oxide, $Cu2HO = CuO + H_2O$.

30. Put into a test tube solution of copper sulphate and a few drops of glycerine (or instead of the glycerine a few grains of neutral potassium tartrate or Rochelle salt). Now add potassium hydrate cautiously; at first a blue precipitate falls, redissolved on the addition of more potass. hydrate. Boiling makes no change in the clear blue fluid.

This property of holding the cupric hydrate in solution is shared by many organic compounds, as glycerine, the tartrates, and glucose; but, when boiled, glucose has the peculiar property of abstracting oxygen from the cupric hydrate, forming the red or cuprous oxide Cu_2O.

31. *Trommer's Test.*—To a half-inch of urine in a test tube add an equal volume of potassium hydrate and a few drops of solution copper sulphate. Heat just to first signs of boiling. If sugar is present, yellow or red cuprous oxide is precipitated.

32. *Glycerine Cupric Test.*—To an inch of potassium hydrate in a test tube add a few drops of copper sulphate and a few drops of glycerine. Boil and add suspected urine by small amounts up to less than one inch in the tube. Sugar will throw out the cuprous oxide, red or yellow.

For convenience and simplicity, the copper sulphate may be dissolved, 28 grains in a fluidounce of pure glycerine, and a few drops used as above.

This *Glycerine Cupric* solution keeps indefinitely and takes the place of two different fluids.

33. *Approximative Method for Bedside Estimates.*—In the absence of finely-graduated instruments, such as standard burettes, it is at times of value to use the ordinary apothecaries' vessels. The results obtained by this method are approximative only.

It is found that, boiled in alkaline solution, one molecule of grape sugar ($C_6H_{12}O_6 = 180$) will reduce to cuprous oxide five molecules of crystallized copper sulphate ($CuSO_4 + 5H_2O = 249.5 \times 5 = 1247.5$). Therefore to make a copper solution in which we shall have sufficient copper sulphate in each fluid drachm to be exactly equal to ½ (0.5) grain of sugar—

$$180 : 1247.5 :: 0.5 : x = 3.47.$$

Dissolve 3.47 grains $CuSO_4$ in a drachm (27.76 grains in the fluidounce) of glycerine; then 15 minims (1 c.c) will be reduced by ⅛ grain of glucose.

Method.—Put 15 minims (1 c.c.) of glycerine cupric solution in a test-tube with 30 minims (2 c.c.) of liquor potassæ and 30 minims (2 c.c.) of water.

Boil, and add 30 minims or drops of urine, and boil again a few seconds.

If the blue color is entirely lost, then there are at least 2 grains of sugar in the fluidounce, or 0.4 per cent.

If the blue color remains, there is less than 2 grains to the fluidounce.

Again, mix 15 mimins of glycerine cupric solution with 30 minims of liquor potassæ and 30 minims of water. Boil, and add 1 drop of the urine, and boil again for a moment. If the blue color is discharged, then there are at least 60 grains to the fluidounce (12.0 per cent.)

If the color is not discharged, add 1 drop more and boil. Entire loss of color indicates at least 30 grains to the fluidounce (6.0 per cent.)

If any color remains, add one drop more and boil. Entire loss of color denotes at least 20 grains to the fluidounce (4 per cent.).

If the amount of urine is insufficient to decolorize, increase it drop by drop, boiling after each addition until there is a sufficient amount to exactly discharge all the blue color. By dividing 60 by the number of drops used, we get the least number of grains of sugar to the fluidounce able to reduce the given quantity. If the amount is very large, the urine may be diluted with an equal volume of water, and then by dividing 120 by the number of drops used, we get the least number of grains to the fluidounce.

34. *Fehling's Test.*—To obviate some of the defects of Trommer's test, Fehling's solution is employed. As it does not keep well, it is best to make it in two parts, not to be mixed until the time of use.

A. Take copper sulph. 34.64 gms. and water enough to make 500 c.c. Mix.
B. Pure Rochelle salt, 173 gms.
Sol. sodium hydrate (S. G. 1.33) 100 c.c., and water enough to make 500 c.c. Mix.
For use, mix equal volumes of A and B, and thus make Fehling's solution.

35. *Method.*—Boil in a test tube 1 inch of Fehling's sol. While hot, add urine and bring to a boil again. If no red or yellow color appears, add more urine until the total volume is 1¾ inches. Again raise to boiling point and set aside. If yellow or red cuprous oxide appears the urine is saccharine.

Precautions.—Let the volume of urine be less than that of the test solution. Heat up to the boiling point without continuing the boiling.

If these precautions are not observed, high-colored, acid, dense urines may reduce the copper sulphate without the presence of sugar.

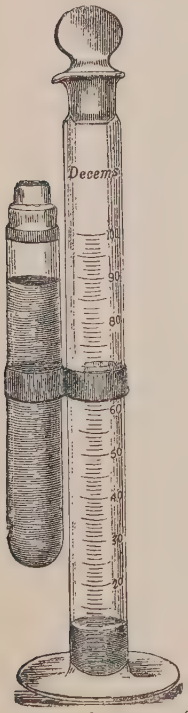
36. *Volumetric Estimation.*—Measure into a capsule 10 c.c. of Fehling's solution and 40 c.c. of water. Heat over wire gauze to boiling, and then from a burette containing a mixture of one volume of urine and nine of water, slowly let it run into the capsule until the color of the test solution disappears. Note the number of c.c. used, and then repeat the experiment with fresh materials to see if a less amount will not answer.

Thus 10 c.c. of standard solution is decolorized by 0.05 gm. glucose. If 8 c.c. of dilute urine were used, then of the undilute 0.8 c.c. = 0.05 gm. glucose. If 1500 c.c. were voided in the day, then 0.8 : 0.05 : : 1500 : x = 93.75 gms. To calculate per cent. 0.8 : 0.05 : : 100 : x = 6.25. Parts per cent. multiplied by 4.55 give grains to the fluidounce.

37. *Pavy's Ammonia-cupric Process.*—This is easy of application, and shows the end of the reaction more definitely than Fehling's method, which it closely resembles. Pavy's solution differs from that of Fehling in containing ammonia, which dissolves the cuprous oxide as soon it is formed, yielding a colorless solution. There is no reddish precipitate to cloud the fluid and make the end point uncertain. The standard reagent is prepared as follows: Dissolve 356 grains of Rochelle salt and the same weight of caustic potash in distilled water; dissolve separately 73 grains of recrystallized cupric sulphate in more water with heat. Add the copper solution to that first prepared, and when cold add 12 fluidounces of strong ammonia (sp. gr. 0.880), and distilled water to 40 fluidounces. The process is as follows:

Dilute 10 c.c. of this standard solution—equivalent to 5 milligrammes of glucose—with 20 c.c. of distilled water, and place in a 6 or 8 oz. flask. Attach this by means of a cork to the nozzle of an ordinary burette, filled previously with the urine diluted 1 part with 9 of water. Boil the blue liquid in the flask resting on wire gauze. Allow the urine to flow into the boiling solution slowly until the blue color is exactly discharged. The number of cubic centimetres used will contain 5 milligrammes of glucose. The urine should be diluted to such an extent that not less than 4 or more than 7 c.c. are required to decolorize the solution, and the proportions necessary will be found to vary from 1 part of urine in 2½ to 1 in 30 or 40. If you wish the percentage of sugar, multiply 0.005 by 100, and divide the product by the number of cubic centimetres of dilute urine employed. The figure thus obtained, multiplied by the extent of dilution—*i. e.*, if there is 1 of urine in 10, multiply by 10—gives the required percentage. The number of grains per fluid-ounce can be obtained by multiplying the percentage by 4.55.

FIG. 15.



Picro-saccharometer of G. Johnson.

38. *Johnson's Picro-saccharometer.*—This test is based on the observation that glucose, in the presence of potassium hydrate, will reduce a bright-yellow solution of picric acid to a red solution of picramic acid. *The value of this change for qualitative testing is much impaired by the fact that normal urine will cause some degree of redness, owing to the creatinine it contains.* In quantitative work some allowance can be made for this in making a standard of color. The test is found practicable by students, and for comparative and clinical purposes may be depended on. The depth of the red color will correspond to the amount of glucose plus the effect of creatinine, which latter is usually equal to that of a solution of glucose containing 0.1 per cent. If albumin is present, the urine is simply made turbid and can be easily cleared by filtration. As a standard of red color, a solution of ferric acetate can be made by the following formula more easily than by Johnson's formula, which uses ingredients of a strength peculiar to the British Pharmacopœia :—

℞. Liq. ferri chloridi, U. S. P., ... f℥j
Ammon. carb., ... ℥j
Ac. acetic., ... f℥v
Aq. destil., ... ad . . . . . f℥iiss. M.

This is put into a stoppered "weighing bottle," which is clamped to a graduated "mixing bottle" of the same diameter and of 100 c.c. capacity, or two cylindrical decimal graduates of equal size may be used.

It is best to standardize the color solution by comparison with that of a tested solution of glucose of known strength, made by rubbing up *one grain of crystallized glucose in a fluidounce of urine.* By this means we practically make allowance for the effect of the amount of creatinine in normal urine. In diabetic urine the proportion of creatinine would be in most cases reduced. If the color of the ferric solution is not deep enough, add a few drops of liquor ferri chloridi, or if too deep dilute it with water. In the intervals of use the apparatus should be kept in the dark to prevent the bleaching action of sunlight. If constantly employed, the color solution should be renewed or standardized every month. Johnson's method is to mix the suspected urine as follows:—

Take of urine, ... f℥j
Liq. potassæ, ... f℥ss
Sol. picric acid (gr. 5.3 to f℥j), . . ♏xl
Water, ... ad . . . . . f℥iv.

Mix; put in a test tube, mark the level of its surface with a file scratch, boil sixty seconds, add a little water up to the mark, and cool. Measure into the graduated cylinder ten parts of this deeply-colored urine. If it is of the same depth of color as the standard, then the ten division denotes 1 grain of sugar to the fluidounce. If of greater depth, add water by small installments, shaking the bottle until it is like the standard.

For every ten division of the diluted urine there is one grain of sugar to the ounce. If it measured 45, then there are 4.5 grains to the fluidounce. If the sugar is very abundant, *i. e.*, over 5 grains to the ounce, dilute the urine 1 part in ten. Then each ten division stands for 10 grains, and 45 would equal 45 grains.

39. To simplify the measurement of the ingredients of the urine mixture, it is customary in Jefferson Medical College laboratory to use but one delivery pipette of 5

c.c. This requires some change in the strength of solutions employed; the liq. potassæ should be of lower specific gravity and the picric acid solution weaker, as stated in the following formula.

Take of urine, ... 5 c.c.
Liq. potassæ (S. G. 1.036), . . 5 c.c.
Sol. picric acid (gr. 3.5 to f℥j), 5 c.c.
Water, ... 5 c.c.

M. Boil sixty seconds, measure and dilute precisely as with the mixture given above.

Having found the number of grains to the fluidounce, the change to parts per cent. is made by multiplying with the factor 0.219.

In all the reduction tests the reading is usually higher than is strictly accurate, owing to the presence of uric acid and creatinine. The aggregate proportion of reducing bodies in normal urine is relatively so small that for clinical purposes it may be disregarded. It is asserted that the reducing power of normal urine upon cupric oxide and picric acid is due to uric acid and creatinine. According to Johnson, both can be removed by adding to the amount of urine to be used $\frac{1}{20}$ its volume of cold saturated solution of sodium acetate, and then $\frac{1}{4}$ its volume of a cold saturated solution of corrosive sublimate. After filtration, should the urine reduce picric acid, then sugar has done the work.

40. *Fermentation Test for Quantity.*—Roberts' Differential Density Method. Take the specific gravity of the urine and record it; mix well urine enough to float the urinometer, say 4 fl. ozs. (120 c.c.) with half a cake of Fleischman's Yeast; set aside in a warm place (mantelpiece) for 24 hours. After the fermentation take specific gravity again and subtract from that taken before. Each degree of the remainder represents one grain of glucose to the fluidounce. Multiply by 0.219 to get percentage.

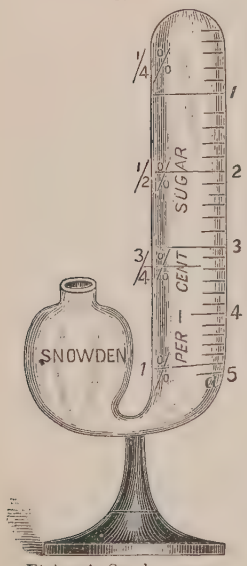
Thus:—Spec. Grav. before fermentation, 1035
" after " 1015

1035 — 1015 = 20 degrees of density lost, or 20 grains of sugar to the fluidounec.

This test is very easily performed and conclusive as to the presence of sugar, though it is not absolutely accurate as to quantity.

41. *Fermentation Saccharometer.*—None of the urinary substances which give reactions of reduction resembling those of sugar, are susceptible of the vinous fermentation. When sugar is present and is mixed thoroughly with brewers' yeast and kept in a warm place for a day, it will ferment, giving off bubbles of carbon dioxide and forming alcohol by the following reaction :—

Fig. 16.



Einhorn's Saccharometer.

$$\underset{\text{Glucose.}}{C_6H_{12}O_6} = \underset{\text{Alcohol.}}{2C_2H_6O} + 2CO_2$$

The fact of fermentation can be shown by collecting the carbon dioxide gas evolved. Fill a test tube with the urine to which yeast has been added, and invert it over a saucer containing some of the same mixture. Set aside in a warm place, and if after 24 hours gas has accumulated it proves the presence of sugar. A convenient vessel for this test is shown in Fig. 16. It is tipped on the side, filled with the urine and yeast mixture, 10 c.c., and kept in a warm place. In 24 hours the gas collects in the upright tube, which is graduated to read from 0.25 to 1 per cent., or from 1 to 5 c.c. It will not show sugar when present to a less amount than 0.1 per cent., and owing to the varying solubility of the gas in urines of variable reaction and density it cannot be considered accurate.

Indigo Carmine Test.—A blue solution of sodium sulph indigotate (indigo carmine) made alkaline with sodium carbonate and boiled with saccharine urine, changes from blue to violet—red—straw-yellow. Cooling, or even agitation, will cause the restoration of the blue color; whereas, if the bleaching is due to strong alkalies, the color will not return. A very handy and reliable form for the test is that adopted by Parke, Davis & Co. in their pellets, each of which contains the indigo carmine and the sodium carbonate combined. The solution does not keep well.

42. *Method.*—Put in 30 minims of water one pellet of indigo carmine and sodium carbonate; heat gently to

solution ; let fall from a pipette *one drop* of urine and boil quietly. A change to red or yellow indicates sugar.

Precaution.—If more than one drop is added there is a chance that a reaction will occur from healthy urine.

43. *Fischer's Phenyl-hydrazin Test.*—Into a test tube put nearly half an inch of dry phenyl-hydrazin hydrochlorate, add pulverized sodium acetate an equal volume, and then fill the tube one-half with the urine. Agitate until the sodium acetate is dissolved, gently heating up to the boiling point, and finally boil for 30 seconds. Set aside about 20 minutes for the sediment to fall, remove a portion of the sediment to the slide of a microscope and examine with a power of 200 diameters.

If sugar be present, needle-shaped crystals of phenyl-glucosazon have formed, as yellow branching sprays, or sheaves, or radiating stars. When the glucose is present in very small amounts it may require 48 hours for the fine crystals to fall in sufficient quantities for recognition. The red globules and large yellow plates often seen are not significant. Geyer found that this test gave positive results with normal urine, and hence considers it unreliable. It has proved of so little practical value that physicians seldom resort to it.

Import of Glycosuria.—If glucose is detected by any of the tests given above it is pathological. If abundant and *persistent*, and associated with excessive drinking and eating on the part of the patient, who is emaciating at the same time, it denotes typical diabetes mellitus. The urine in this disease is usually pale, of a fruity odor and very abundant. It may amount to over two gallons or 10.000 c.c. daily, and, notwithstanding the large volume, the presence of sugar makes it dense, the specific gravity ranging from 1025 to 1045.

Temporary, slight glycosuria may follow an excessive saccharine diet, and reducing substances simulating glucose may appear in urine after the administration of chloroform, ether, chloral, amyl nitrite, turpentine, salicylic acid, benzoic acid, glycerine, camphor and carbolic acid; also after poisoning from strychnia, morphia, arsenic, phosphorus or carbon monoxide. These reducing derivatives do not, however, undergo fermentation, as glucose would.

It may be *incidental* to various diseases of the brain and spinal cord, and accompany phthisis, pneumonia, asthma, cirrhosis, cholera, and even be intermittent, like malarial paroxysms.

ALBUMIN.

In addition to the blood serum and blood globulin, which are congeners and have the same serious significance, other proteid principles of different import may occur in the urine, such as mucin, peptone, hemialbumose. The albumin of the blood serum occurring in the urine is abnormal, and is nearly always evidence of grave disease. It is detected by the use of heat and of various reagents which throw it out of solution as a white precipitate. In case of turbid urine, it is necessary to filter the urine clear before testing. If the urine is turbid from urates filtration may be dispensed with if the boiling test is used; gentle heat first causes the disappearance of the urates, and as the temperature approaches the boiling point the albumin coagulates.

44. *Nitric Acid Test, Heller's Method.*—Put 1 inch of urine in a test tube and gradually run ¼ inch nitric acid down the side while the tube is inclined, so as to prevent mixing. The acid is heavier and forms a bottom layer. Wait some minutes for the appearance of a white belt or zone *at the line of contact* of urine and acid. This is a coagulum of albumin. Although this is not the most delicate test, it is highly convenient, and albuminuria is rarely serious unless decided enough to be shown by this "contact" method.

In dense urines a haziness may appear in the upper part of the urine, due to acid urates. Heat will clear up the urate cloud, whereas albumin will remain undissolved. In case of doubt, try another sample diluted one-half.

45. *Roberts' Nitric Magnesian Reagent.*—This reagent has been devised in order to obviate objections to handling nitric acid, which, if undiluted, is a corrosive liquid not conveniently portable. It can be kept in a cork-stoppered bottle and does not stain the hands. To 5 volumes of a filtered saturated solution of magnesic sulphate, prepared by dissolving 10 parts of the salt in 13 parts of distilled water, add 1 volume of strong nitric acid. A couple of drachms of bright filtered urine is allowed to float on an equal quantity of this solution in a test tube; care being taken that the contact-line is sharply defined. In a period of time varying from a few seconds to

a quarter of an hour, according to the amount of albumin present, a delicate opalescent zone forms at the point of junction, and if mucin also is present a more diffused haze *higher up* in the urine. Special attention should be given to the position of the opacity. In some concentrated urines a belt of urates will appear a little distance above the line of demarcation; but these dissolve on warming.

46. *Boiling Test.*—First test the reaction. If acid, proceed at once to apply heat. If the urine be alkaline, then to a tube containing 3 inches of urine add one drop of acetic acid. Heat the upper half to boiling. If contrasted with the lower half it has a turbid appearance, then albumin is present. Albumin coagulates in acid fluids at 70° C. (160° F.), but remains dissolved if the urine is alkaline. Serum albumin and its associate globulin are the only proteids that coagulate at this temperature.

Precaution: Sometimes when acetic acid is added to a urine already acid, the albumin is converted into acid albuminate, a form non-coagulable even on boiling. The acetic acid should not be employed except in an alkaline urine and even then only a drop, just enough to neutralize the reaction or make it slightly acid.

47. *Rough Estimation.*—The urine acidulated by a drop of acetic acid is boiled for several minutes and set aside for 24 hours. White flakes fall in a well-defined layer, the depth of which may be expressed as a fraction of the whole fluid in the tube, thus: it is $\frac{1}{10}$ or $\frac{1}{4}$ albuminous layer.

48. *Picric Acid Test.*—A saturated solution is prepared by dissolving 6 grains of recrystallized picric acid in a fluidounce of water with heat, and decanting the clear solution. Some of the urine is rendered perfectly clear by filtration; the tube is held inclined, and an equal volume of the picric acid solution is gently poured down

the side so as to form a top layer. In the presence of albumin a more or less distinct haze is produced, which on heating to the boiling point is rather intensified than otherwise.

Fig. 17.

Fallacies: Peptones, if present, yield a similar haze, and quinine or other alkaloids a more or less crystalline precipitate; but in both these cases the opalescence is completely dissipated by heat. Mucin, an important constituent of some urines, is also coagulated, but much more slowly than the other proteids.

Picric acid is a sensitive test for albumin, but its clinical value is impaired by the fact that it gives a reaction under conditions not serious.

49. *Esbach's Albumimeter.*—The only accurate way of estimating the quantity of albumin is to coagulate the albumin by boiling the acidulated fluid, then, after filtration, drying and weighing the precipitate.

It is sometimes desirable to determine the proportion present at intervals, so as to study the progress of a case. For this comparative and clinical purpose, the easy method of Esbach is to be commended. A tube graduated like that in Fig. 17 is to be employed. If care is taken that the urine shall be previously neutral or acid, Johnson's saturated solution of picric acid may be employed. Dr. Esbach's test solution is prepared by dissolving 10 grammes of picric acid and 20 grammes of citric acid in 900 c.c. of boiling distilled water, and then adding when cold sufficient water to yield 1 litre. The citric acid is only employed

for the purpose of maintaining the acidity of the liquid, and is not essential except for urines decidedly alkaline.

The filtered acid urine is poured into the glass tube up to the mark U, and then the reagent is added till the level of the liquid stands at R. Mix the liquids thoroughly, without shaking, by reversing the tube a dozen times, close with a cork, and allow it to stand upright for twenty-four hours. The height at which the coagulum then stands, read off on the scale, will indicate the number of parts per thousand, or grammes of albumin in one litre. This divided by ten gives the percentage. If the coagulum stands above the mark 4, try again after first diluting the urine with one or two volumes of water, and then multiply the resulting figure by 2 or 3, as the case may be. If the amount of albumin be less than 0.5 gramme per litre it cannot be accurately estimated by this method.

50. *Acetic Acid and Ferrocyanide Test.*—Hydroferrocyanic acid yields a precipitate immediately in the presence of much albumin, and, if traces only are present, in the course of a few minutes. To apply the test, strongly acidulate with acetic acid, and then add a few drops of recently prepared potassic ferrocyanide solution. This is one of the most delicate tests known, but is open to the objection that it precipitates hemialbumose, though it does not affect peptone.

Trichloracetic Acid.—This test is said to have more than six times the delicacy of metaphosphoric acid and more than three and a half times that of nitric acid. It may be used in the crystalline form or in saturated solution. If used in the solid form, it is dropped into the specimen and a turbidity results if albumin is present. In the liquid form it is best used by superposition in the same way as Heller's nitric acid test. The white ring which forms at once is characteristic and unobscured by the colored zone of oxidized pigments as in the nitric-acid test.

Acidulated Brine Test.—A saturated solution of sodium chloride with 5 per cent. of ac. hydrochloric. dilut. may be used by the contact method. It sometimes gives a cloud with normal urine.

51. *Potassio-Mercuric Iodide or Tanret's Test.*—The reagent is composed of mercury bichloride, 1.35 gms.; potassium iodide, 3.32 gms.; acetic acid, 20 c.c.; distilled water enough to make 1000 c.c. Used by contact, it gives an opacity with all the proteids, and some reaction with a large proportion of normal urines. On this account, like *sodium tungstate* and *metaphosphoric acid*, it is open to fatal objections as a clinical test.

Import of Albuminuria.—If serum albumin is persistently present, and when deposited by the boiling test makes a layer as thick as ½ of the column of fluid, it indicates Bright's disease. It appears transiently in febrile diseases, blood poisonings, pregnancy, epilepsy. It is found in poisoning from lead and arsenic. Traces sometimes appear in adolescents from trivial causes. To make the diagnosis of Bright's disease conclusive, when only small amounts are present, tube casts must also be found, or some of the general symptoms, such as anæmia, cardiac hypertrophy, dropsy and eye-lesions.

PEPTONE

Peptone may be found in the urine with or without albumin.

52. Apply the nitric acid boiling test, § 46; if a negative result follows, then test a fresh portion with acetic acid cold. A cloudiness would justify the suspicion of peptone.

53. *Biuret Test.* — Having got rid of the albumin by boiling, after acidulating with nitric acid and then filtering, pour slowly about a drachm of this clear urine down the side of a test tube which contains an equal quantity of Fehling's solution.

At the line of contact of the two fluids a cloudy belt of phosphates will form, above which, if peptone be present, a rose-pink halo will appear. Hemialbumose gives the same result. The presence of albumin imparts a violet hue.

Import.—Peptone is present mainly as a product of the breaking up of inflammatory effusions by suppurative process in various tissues and organs.

HEMIALBUMOSE

is a mixture of proteids intermediate between albumin and peptone. It appears in the urine under like conditions with peptone. It will form a

cloud with cold nitric acid, but clears up on heating. It is thrown out by acetic acid and potassium ferrocyanide.

HÆMATURIA.

If the blood of hæmaturia is derived from the *kidney*, it is well mixed with the urine, to which it imparts a smoky appearance, and microscopic tube casts can usually be found. If from the *ureter*, large vermicular clots may be found. If from the *bladder*, the blood is usually abundant and bright red, with stringy clots.

54. *Microscopic Appearances.*—The most satisfactory test for blood is the presence of the peculiar corpuscles.

These corpuscles in ordinary acid urine retain their bi-concave shape for several days (Fig. 19). If the urine is dense, they become crenated (Fig. 18). When the urine is alkaline from ammonia, as in cystitis, the red corpuscles are shriveled and accompanied by numerous leucocytes.

HÆMOGLOBINURIA.

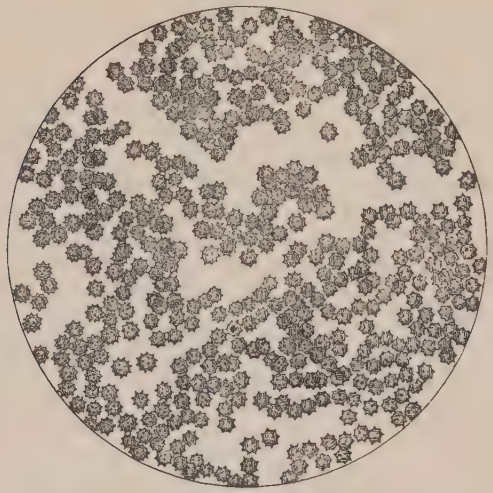
When the blood-coloring principle hæmoglobin is excreted in the urine, diffused and not corpuscular, other tests than the microscopic must be resorted to. These tests indicate hæmoglobin, either with or without the corpuscles. The microscope will serve to exclude the corpuscular element.

55. *Test.*—To a suspected sample add a drop of acetic acid, and boil. A red coagulum indicates hæmoglobin.

56. *Almen's Test.*—To suspected urine add a few drops of tr. guaiac, freshly made. A white or greenish turbidity ensues. Shake well and add a few drops of solution of hydrogen peroxide or of an old sample of oil of turpentine. Hæmoglobin will slowly change the color of the precipitate to blue.

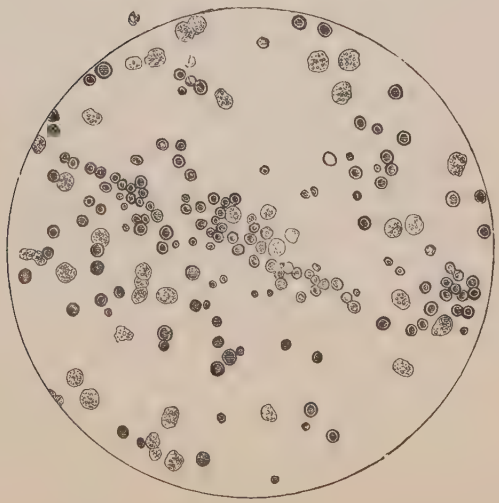
Turpentine slowly absorbs oxygen, and after standing on the chemist's shelves for some time will act for this test as an ozone solution. As saliva or potassium iodide will have the same effect as blood, these must be excluded before a positive conclusion can be reached.

FIG. 18.



Crenated red blood corpuscles in the urine × 350.

FIG. 19.



Colored and (*a*) colorless blood corpuscles of various forms.

Import.—*Hæmaturia* indicates hemorrhage from some part of the genito-urinary tract.

Hæmoglobinuria occurs in various diseases, attended by "a dissolved state of the blood," as pyæmia, scurvy, typhus, purpura, poisoning from hydrogen arsenide, phosphorus, carbolic acid, chloral, potassium chlorate. There is a periodical form, the pathology of which is obscure.

PYURIA.

As pus is an albuminous fluid, its presence will cause the urine to respond to the tests for albumin. In addition, it contains white corpuscles, which are spherical, granular and opaque. (Fig. 4.) The mucus corpuscle is indistinguishable from it, but mucin will not become hazy with the cold nitric acid test for albumin.

57. *Donnè's Test.*—Let the suspected urine stand several hours for the pus to settle, and decant the clearer part. Into the deposit put a piece of potassium hydrate and stir with a glass rod; pus will grow thick, tough and gelatinous, while mucus will form flakes and become thin.

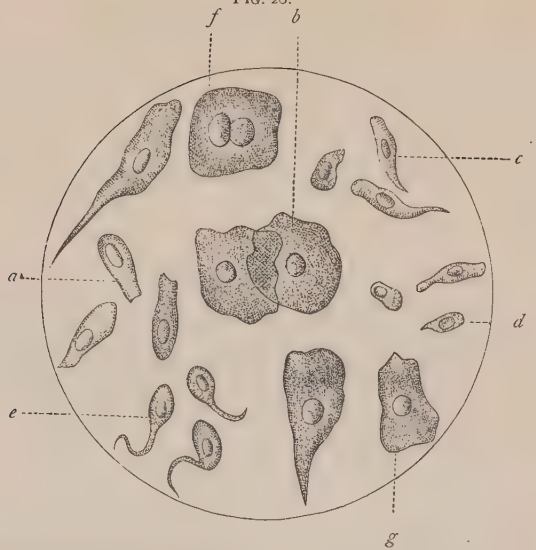
Import.—A sudden appearance of pus in the urine would point to the opening of an abscess. Usually pyuria is an indication of catarrh or inflammation of some part of the genito-urinary tract.

EPITHELIUM.

The mucous membrane of any part of the genito-urinary passages may shed its cells into the urine. While there is a great variety in form, it is usually not difficult to distinguish renal epithelium from that of *extra-renal* origin. The accompanying Fig. 20 will give an idea of the microscopic appearance of the tessellated, the columnar and the transitional cells that appear at times from different parts external to the kidney.

Free renal epithelium is spherical, granular, nucleated, with a very faint wall or no wall whatever. Its character is easily made out by the fact that careful search will always reveal renal tube casts in the same sample.

FIG. 20.



(*a*) Epithelium from the human urethra; (*b*) vagina; (*c*) prostate; (*d*) Cowper's glands (*e*) Littre's glands; (*f*) female urethra; (*g*) bladder.

FIG. 21.

(*a*) Blood cast; (*b*) granular cast with epithelial cells imbedded; (*c*) amyloid or wax cast.

TUBE CASTS.

When either albuminuria or hæmaturia attends diseases of the kidney structure the deposit in the urine will be found to contain microscopic casts of the tubules. They may be found in cases in which neither albumin nor blood can be detected by ordinary testing. Sometimes they are made of coagulated fibrin, sometimes of mucoid exudation, sometimes of the plastic material of broken-down epithelial cells.

I. *Epithelial casts* are composed of plastic matter with cells imbedded. They mark the process of desquamation (Fig. 21–*b*).

FIG. 22.

Hyaline casts.

II. *Hyaline casts* are either transparent, soft, and very delicate in outline, or transparent, well-defined and brittle. The former variety is called *mucous* (Fig. 22), and is not always an indication of nephritis; the latter, *waxy* (Fig. 21–*c*), is evidence of serious nephritis.

III. *Granular casts* are opaque and yellowish. They are mucoid or waxy, or cellular material which has undergone a granular change. They often contain cells and fat globules (Fig. 21–*b*).

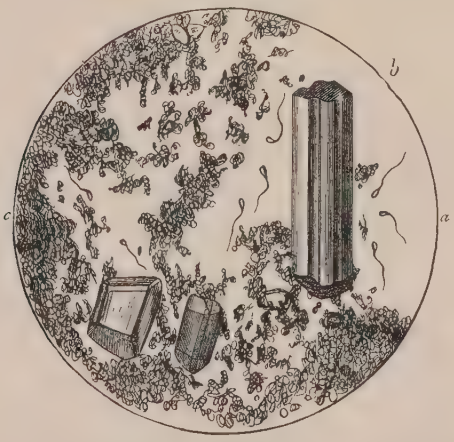
IV. *Fatty casts* are those which carry free fat globules along with the coagulated matter. They are usually considered proof of fatty degeneration of kidney.

v. *Blood casts* are composed of coagulated blood with corpuscles imbedded (Fig. 21–*a*). They show that the hæmaturia originates in the secreting structure of the kidney.

SPERMATOZOA.

These may be present in the urine voided after coitus or an involuntary nocturnal emission of semen, or be an indication of spermatorrhœa. They

FIG. 23.



(*a*) Spermatozoa; (*c*) amorphous calcium carbonate; (*b*) crystalline magnesium phosphate.

form a viscid, mucus-like deposit, which, under the microscope, is seen to be composed of ciliated bodies, like a tadpole, $\frac{1}{600}$ inch long. In the urine they are motionless and resist decomposition for days.

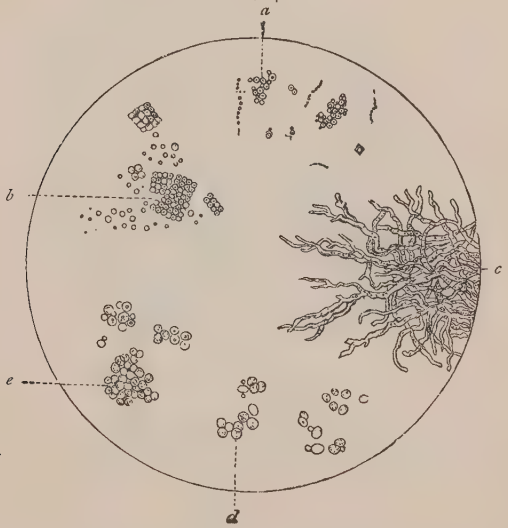
CHYLURIA.

Under very rare conditions the urine contains chyle. It then presents the appearance of milk forming a cream at the surface, and owing to the fibrin of the chyle it may coagulate spontaneously like a white jelly. It responds to the various tests for albumin. If agitated with ether it clears up, the fatty matter is dissolved and separates as a layer at the top. Under the microscope the fat appears in a molecular state as small, bright granules, soluble in a drop of ether. The same substances appear in the urine when the filaria sanguinis hominis is in the blood.

MICROÖRGANISMS.

After emission, the urine may become the medium for the development of many low forms of life proceeding from germs that get into it from the air of the containing vessels. Various *moulds* and the *yeast fungus* (Saccharomyces cerevisiæ) are commonly met with in putrid or saccharine urine after several days. *Sarcinæ* in quantities have been found in urine at the time of discharge, probably produced in the bladder. Local symptoms are supposed to be due to their growth. These vegetations appear in microscopic cubes composed of distinct particles.

FIG. 24.



(*a*) Micrococci in short chains and groups; (*b*) sarcinæ; (*c*) fungi from acid fermentation; (*d*) yeast cells from diabetic urine; (*e*) mycelium of a fungus.

Bacteria not only cause the ordinary decomposition of stale urine outside but sometimes set up like processes inside the bladder. It is likely that this form of bacteria gets access to the urine in the urinary passages by means of dirty instruments, such as catheters and sounds. They may steal down the urethra in the inflammatory products of old gonorrhœa. If, now, the urine is detained in the bladder, these organisms set up the ammoniacal fermentation which soon induces cystitis.

URINARY DEPOSITS.

Beside the different constituents of urine, healthy and morbid, which are seen in the deposits, extraneous substances are sometimes found. Some of these microscopic bodies, such as hair, or fibres of cotton and linen, may be mistaken for tube casts. A dirty vessel is often responsible for the presence of oil, starch, wood, etc. *Healthy urine* may form, at or near the bottom, a light cloud of epithelial débris suspended in mucus. But if on emission an *acid* urine is *turbid* throughout, excess of mucus or pus or blood is present. If an *alkaline* urine is *turbid*, then the precipitated phosphates may be the cause.

58. A *specimen* may be obtained for examination by allowing the urine to settle, and then by means of a pipette a drop or two of the sediment is conveyed to the glass slide and covered with a thin glass disk.

Examine with a ¼-inch objective, or any combination giving a power of 200 to 300 diameters.

If *amorphous*, it may be urates or phosphates.

1. Put a few drops of the deposit into a test tube with some urine and *warm* it; the urates will dissolve, the phosphates not.
2. To another portion add any acid, such as nitric or acetic; the earthy phosphates will dissolve.

If minute *globular particles* with bright centres, it may be milk, chyle or fat.

Shake some of the urine with ether; if chylous, its milky appearance will disappear and the fat separate as a top layer.

If *crystalline*, it may contain one or more of the substances already described and figured: uric acid, Fig. 6; calcium oxalate, Fig. 9; triple phosphate, Fig. 4; calcium phosphate; cystin, Fig. 10; leucin and tyrosin, Fig. 11.

If *organized* bodies, they may be epithelial cells, Fig. 20; mucus or pus, Fig. 4; blood, Fig. 19; spermatozoa, Fig. 23; yeast fungus, mould, sarcinæ, bacteria, Fig. 24.

If a microscope is not at hand, the nature of an unorganized deposit may be determined by the following method:—

59. *Rough Tests for Common Unorganized Deposits:—*

1. Warm the deposit with some urine.

   It dissolves = urates.

   It does not dissolve = phosphates, or calcium oxalate, or uric acid.

2. Warm a fresh portion with acetic acid.

   It dissolves = phosphates.

   It does not dissolve = calcium oxalate, or uric acid.

3. Warm a fresh portion with hydrochloric acid.

   It dissolves = calcium oxalate.

   It does not dissolve = uric acid.

60. PLAN FOR PRACTICAL EXAMINATION.

1st. Ascertain from patient the total daily discharge.

2d. Note if the color and odor are normal. If deep yellow, green or brown, test for biliary pigment, § 2. If red or chocolate, test for hæmoglobin, § 55–56.

3d. Determine the reaction with litmus paper.

4th. Take the specific gravity and calculate the solid urine.

5th. Having allowed the urine to settle, carefully decant the clear part into a test tube until it is half full, and examine for albumin by boiling the upper part. One drop of acetic acid must be added if the reaction is not acid. If the flakes formed are white, pure albumin is present. If greenish, probably bile pigment is the cause. If red-brown, there is probably blood. After 24 hours, note the height of albuminous layer. Confirm by test with cold nitric acid.

6th. Test for glucose by Fehling's and by Böttger's method.
Determine the amount.

7th. Estimate if the chlorides are absent or much reduced.
Determine the amount.

8th. Estimate the amount of urea.

9th. Describe the naked-eye characters of the deposit, and also the microscopic appearances. If amorphous, and the urine is acid, then the deposit is composed of mixed urates; if the reaction is alkaline, then the deposit is phosphatic. To make sure, test for urates and phosphates, § 59.

URINARY CONCRETIONS.

About 80 in 100 of all urinary concretions are made of *Uric Acid and Urates.* Next in frequency are the *Calcium Oxalate,* or mulberry calculi. The rarer primary constituents are *Blood Concretions, Cystin, Xanthin, Calcium Phosphate, Calcium Carbonate.* Secondary to any of these there may appear at the last stage in the history of a calculus a deposit of *Mixed Phosphates.* These form a white layer, precipitated upon the calculus as a result of ammoniacal decomposition in the urine.

As two or more deposits of different composition may alternate in the formation, in *examining* a concretion it should be sawed through the middle, so as to expose its concentric layers. A small portion of each distinct layer should be examined by the following method :—

61. CASUAL ANALYSIS OF CONCRETION.

First burn a portion of the powder on Platinum foil in a Bunsen burner or blow-pipe flame.

A. *It chars greatly, leaving but little ash.*
= (Uric acid, urates, cystin, xanthin, blood.)
It gives murexide reaction. See page 23.
= (Uric acid or urates.)
It dissolves in boiling water.
= (Urates.)
It does not dissolve in boiling water.
= (Uric acid.)

Cystin and xanthin are very rare; the first can be recognized by test on page 26.

B. *It chars very slightly, leaving very much ash.*
= (Phosphates, oxalate, carbonate of calcium.)

1. Treat a fresh portion with dilute hydrochloric acid.
It is soluble with effervescence. = (Carbonates are present.)
" " *without* " = (Phosphates or oxalate calcium.)

1. Treat with acetic acid.
If *it is soluble without effervescence*, then it is phosphatic; it fuses into a bead on platinum foil (mixed phosphates); it does not fuse (calcium phosphate).
If *it is insoluble*, then it is calcium oxalate, and when calcined on platinum, leaves an ash that turns red litmus blue, or effervesces with HCl.

REAGENTS AND APPARATUS FOR URINALYSIS.

Acid, Nitric.
" Sulphuric.
" Hydrochloric.
" Acetic. (Sp. Gr. 1.048.)
" Picric, 5 gr. to ℥j.
Ammon. Hydrate, 20°.
Potass. " (normal).
Silver Nitrate (standard).
Copper Sulphate. (1 in 10.)
Potassium Chromate, neutral. (1 in 10.)
Magnesia Mixture ($MgSO_4$ 10; NH_4Cl 10; Aq. Ammon. fort. 10; Water 80.)
Sodium Hypochlorite (Labarraque).
Barium Chloride (standard).
Fehling's Solution.
Glycerin.
Litmus Test Papers.

Burette with pinch cock.
Pipette, 5 c.c.
Capsule, 50 c.c.
Graduated Cylinder, 50 c.c.
Urinometer.
Ureometer.
Urinary Pipette.
Glass Rod.
Blowpipe.
Watch Glasses.
Wire Gauze.
Platinum Foil.
Funnel.
Filter Paper.
Tripod.
Bunsen's Burner.

REAGENTS FOR TOXICOLOGY.

In addition to the reagents and apparatus mentioned above, the following solutions are required for the experiments described in the second division of this book.

Acid Oxalic (normal solution).
Calcium Hydrate (Lime Water).
Potassium Iodide. (1 in 20.)
Potassium Bichromate. (1 in 10.)
Ammonium Chloride. (1 in 10.)
Ammonium Carb. (1 in 20.)
Potassium Ferrocyanide. (1 in 10.)
Ferrous Sulphate. (1 in 20.)
Ferric Chloride. (1 in 10.)
Ammon. Sulphhydrate.
Ferrous Sulphide in lumps for making H_2S.

EXAMINATION

FOR

COMMON POISONS.

CLASSIFICATION OF POISONS.

The following simple arrangement of commonly occurring poisons is adopted as suited for the preliminary tests of the practitioner of medicine:—

A. Acids. *Nitric*, HNO_3; *Hydrochloric*, HCl; *Sulphuric*, H_2SO_4; *Oxalic*, $H_2C_2O_4$.

B. Alkalies. *Potassa*, KHO; *Soda*, NaHO; *Ammonia*, NH_4HO.

C. Volatile Matters. *Carbolic Acid*, C_6H_5OH; *Hydrocyanic Acid*, HCN; *Phosphorus*, P; chloroform, $CHCl_3$; chloral, C_2HCl_3O; alcohol, C_2H_5HO.

D. Metallic Poisons, subdivided into—

1. Those precipitated *yellow* or *orange* from an acid solution by hydrogen sulphide, H_2S; *Arsenic, As.; Antimony, Sb.;* cadmium, Cd; tin, Sn.
2. Those precipitated *black* from an acid solution by hydrogen sulphide, H_2S; *Copper, Cu; Mercury, Hg; Lead, Pb;* silver, Ag; bismuth, Bi; platinum, Pt.; gold, Au.

3. Those precipitated by adding ammonia, NH_4HO with ammonium sulphide, $(NH_4)_2S$; *Zinc, Zn;* nickel, Ni; cobalt, Co; iron, Fe; chromium, Cr; manganese, Mn; aluminium, Al.
4. Those precipitated *white* by adding ammonia, NH_4HO and sodium phosphate, Na_2HPO_4; the metals of the alkaline earths; *Barium, Ba;* calcium, Ca; strontium, Sr; magnesium, Mg.
5. Those not precipitated by preceding reagents: the alkaline metals.

E. Vegetable Alkaloids. *Morphine, Strychnine, Atropine.*

ACIDS AND ALKALIES.

The mineral acids and the alkalies corrode the tissues with which they come in contact and impart a strong corresponding reaction to the vomited matters. If alkaline antidotes have been administered the reaction may be neutral, or even alkaline, and if alkaline poison has been treated by vinegar or lemon juice the reaction may be changed to neutral or acid. The acids may have been converted into nitrates, chlorides or sulphates. A complete examination of suspected material in the case of chlorides or sulphates requires an estimation of the *quantity*, to determine if these salts are in excess. As nitrates are not found in the normal viscera or the ordinary gastric contents, their bare presence in notable amounts is very suspicious.

Besides determining the fact of acidity or alkalinity of the vomited matters, it may be necessary to determine the degree. This is done by measuring the amount of a standard test solution necessary to neutralize the sample, using litmus or phenolphthalein as an indicator of the neutral point.

Rules for Volumetric Analysis.—1. Let some of the titrating solution run through the burette to wash it out. 2. Fill the burette to the brim at first, then let it run out into the supply bottle until the upper surface stands at the 0 mark. 3. At the first titration let the flow be drop by drop, making frequent trials with the indicator.

1. *Acidimetry Experiment.*—Measure 1 c.c. of nitric acid, HNO_3, and fill to 50 c.c. with water. Put this dilute

acid into a capsule or beaker and add a few drops of red litmus solution, or two drops of solution of phenolphthalein (1–100 alcohol). Fill a graduated burette (Fig. 2) to 0 with normal solution of potassium hydrate, KHO (56 grams to 1000). Now, let this alkaline solution run, by drops, into the capsule until a violet color appears. Note the c.c. of KHO solution used, and calculate the amount of HNO_3 neutralized by multiplying the c.c. of KHO used with 0.0908. If phenolphthalein be used as indicator, neutralization is denoted by a loss of the red alkaline color.

$$HNO_3 + KHO = KNO_3 + H_2O.$$

Potassium nitrate and water are formed.

Repeat this experiment with acids of different strength.

2. *Alkalimetry.*—This procedure is like acidimetry, using blue litmus as an indicator and a normal solution of oxalic acid (63 grammes to 1000) for testing. Each c.c. = 0.656 gramme of KHO.

Nitric Acid, HNO_3. *Aqua Fortis.*

3. Test reaction with blue litmus paper.

4. Pour HNO_3 on copper slips in a test tube and look for red-brown nitrous fumes, the liquid turning blue.

$$3Cu + 8HNO_3 = 3(Cu2NO_3) + 4H_2O + N_2O_2.$$

Copper nitrate, water and nitric oxide are formed, and nitric oxide in contact with air changes to red fumes of higher oxides.

5. Touch the skin and the clothing with a drop of HNO_3, and note in each case a yellow stain of picric acid, not removed by aqua ammonia.

6. HNO_3 turns a crystal of morphia yellow, but one of brucia red.

7. To dilute HNO_3 or a solution of a nitrate add a green crystal of ferrous sulphate and a drop or two of sulphuric acid. The crystal turns red brown, ferric sulphate being formed.

Acid Hydrochloric, HCl. *Spirit of Salt.*

8. Test with blue litmus paper.

9. Add HCl to a few grains of manganese dioxide, MnO_2 in a test tube. Warm and note that chlorine gas forms and will bleach moist litmus paper held at the mouth of the tube.

$$MnO_2 + 4HCl = MnCl_2 + 2H_2O + Cl_2.$$

Manganese chloride, water and chlorine are formed.

10. Hold side by side the open mouths of bottles of HCl and of ammonia, NH_4HO, observing the white fumes of NH_4Cl formed.

11. Add to dilute HCl or a solution of sodium chloride, NaCl, a drop of silver nitrate, $AgNO_3$. A white precipitate falls, soluble in ammonia but insoluble in HNO_3.

$$NaCl + AgNO_3 = NaNO_3 + AgCl.$$

Sodium nitrate and silver chloride are formed.

Mercurous salts will yield with HCl a white precipitate of Calomel, Hg_2Cl_2, insoluble in HNO_3 and blackened by NH_4OH.

Lead salts give a white precipitate of lead chloride, $PbCl_2$, insoluble in NH_4OH.

12. Put into a test tube a solution of methyl violet, distinctly violet in hue; add a few drops of filtered vomit or other matter; if free mineral acid is present the violet changes to blue, the vegetable acids being without effect or perhaps changing the violet to dirty yellow. HCl will also change Congo-red paper to blue.

13. *Uffelman's* solution to distinguish HCl from lactic acid in gastric contents is made by adding a drop of neutral ferric chloride solution to a mixture of 10 c.c. of a four per cent. solution of carbolic acid and 20 c.c. of water. This steel blue liquid is turned yellow by lactic and butyric acids but not affected by HCl when diluted to the strength of gastric juice (0.2 per cent).

Reactions 12 and 13 are used clinically to determine the presence or absence of HCl in vomit. HCl is nearly always absent from gastric juice in cancer of the stomach. The value of these tests is impaired by interference from peptones, chlorides and phosphates.

Sulphuric Acid, H_2SO_4. *Oil of Vitriol.*

14. Test with blue litmus paper.

15. Put a few drops of H_2SO_4 with a drop of water on a sheet of paper and warm gently until dry; a charred spot appears.

16. To dilute H_2SO_4 or a soluble sulphate, add barium chloride, and a heavy white precipitate falls.

$$H_2SO_4 + BaCl_2 = 2\,HCl + BaSO_4.$$

Hydrochloric acid and barium sulphate are formed.

17. A drop of H_2SO_4 applied to the black lining of the coat causes in time a moist red stain, which is removed by ammonia.

Strong H_2SO_4 when heated with Cu generates colorless suffocating fumes of SO_2:

$$Cu + 2H_2SO_4 = CuSO_4 + SO_2 + H_2O.$$

Oxalic Acid, $H_2C_2O_4$.

18. Oxalic acid added to lime water gives a white precipitate of calcium oxalate.

19. Heat a crystal of oxalic acid in a test tube. It is volatile and condenses in the cool part of the tube.

20. Precipitate a solution of oxalic acid with silver nitrate. It is white, and when separated by filtration and dried, it will disperse in vapor if heated on platinum foil.

21. Repeat the experiment with silver nitrate and divide the white mixture between two test tubes. The precipitate of one will dissolve in nitric acid, that of the other in ammonia.

ANTIDOTES TO THE ACIDS.—*Calcined Magnesia* suspended in milk is the best. If this cannot be had, then prepared chalk, whiting or plaster from the wall, or eggs. For the mineral acids, soap or soapsuds may be used, but they are not antidotes to oxalic acid. The stomach tube would probably injure the corroded tissues.

SHORT SYSTEM OF TESTS FOR THE STRONG MINERAL ACIDS.

Test for each acid in a fresh portion of the sample:—

Add $BaCl_2$; white prpt., insoluble in HNO_3, shows . . . . . H_2SO_4

If Hg_2, Ag, or Pb are present, use $BaNO_3$ instead of $BaCl_2$.

Add $AgNO_3$.

1. White prpt., soluble in NH_4HO and reprecipitated by HNO_3 shows . . . . HCl.

2. Yellowish prpt., insoluble in NH_4HO, shows HI.

HCl may also be present. Filter; to filtrate add HNO_3; prpt. shows . . . . HCl.

Add strong H_2SO_4, then strong, fresh solution of $FeSO_4$, in such a way that the two fluids do not mix; shake then very gently; a brown color at junction shows . . . . . HNO_3.

Add HCl.

1. Effervescence and smell of H_2S shows . H_2S.

2. Effervescence and no smell shows . H_2CO_3.

ALKALIES.

Potassium Hydrate, KHO. *Caustic Potash.*

22. Test with red litmus or turmeric paper.

23. Dilute the KHO with an equal part of water, and add HCl until the reaction is neutral to litmus paper.

$$KHO + HCl = H_2O + KCl.$$

Water and potassium chloride are formed.

24. To this potassium chloride add platinum chloride; a yellow granular precipitate falls slowly. Ammonium salts give a similar result.

$$2KCl + PtCl_4 = PtCl_4.2KCl.$$

A double salt, potassio-platinic chloride, is formed.

25. Heat platinum foil or wire in a Bunsen's burner until the flame is colorless; then touch it with moist KHO and heat again; a violet tint appears. If mixed with yellow, observe through deep blue glass, which shuts out the yellow rays and leaves the violet.

Sodium Hydrate, NaHO. *Caustic Soda.*

26. Test for alkaline reaction.

27. Add HCl to neutralize.

28. Heat NaHO on platinum foil; the flame is colored yellow.

This test is so delicate as to reveal a trace of sodium present almost everywhere.

Ammonium Hydrate, NH_4HO. *Aqua Ammonia.*

29. Note the odor of aqua ammonia or ammonium carbonate.

30. Test the fumes with moist red litmus paper; they turn it blue.

31. Neutralize with HCl; observe the white fumes of ammonium chloride, NH_4Cl, and note that the ammoniacal odor ceases.

32. Boil the last product, NH_4Cl, with KHO; the odor of free ammonia may be perceived.

$$NH_4Cl + KHO = KCl + NH_4HO.$$

Potassium chloride and ammonia are formed.

ANTIDOTES TO THE ALKALIES.—*Vinegar and Water* (dilute acetic acid), lemon juice, oils, butter, milk. Do not use the stomach pump.

VOLATILE POISONS.

Under this head are included carbolic acid, hydrocyanic acid and phosphorus as the most important, and chloroform, chloral, ether, alcohol, nitrobenzene, besides some others of less importance in toxicology. Peculiar odors are distinctive of nearly all the volatile poisons.

ACID CARBOLIC, C_6H_5HO. *Phenol.*

33. Note the odor.

34. A drop applied to blue litmus paper leaves a greasy stain, but does not redden the paper.

35. To a dilute solution of acid carbolic add a few drops of nitric acid and warm gently; it turns yellow from the formation of picric acid.

36. Strong bromine waters causes a whitish-yellow precipitate.

ANTIDOTES.—*Saccharate of lime*, lime water, magnesia, eggs. The stomach pump may be used freely.

ACID HYDROCYANIC, HCN or HCy. *Prussic Acid.*

37. Note the odor of bruised peach kernels.

38. Put HCy or potassium cyanide solution, KCN,

into a test-tube, and add a few drops of acid sulphuric. Cover with a watch crystal or saucer wet with 1 drop of silver nitrate and warm gently. A white spot appears, which is soluble in warm nitric acid.

$$KCN + AgNO_3 = KNO_3 + AgCN.$$

Potassium nitrate and silver cyanide are formed.

39. Cover with another watch crystal wet with potassium hydrate. After a few minutes, touch with ferrous sulphate and with ferric chloride, and finally with one drop of HCl. A precipitate of Prussian blue (ferrocyanide of iron) forms.

40. Cover with another watch crystal or saucer wet with ammonium sulphide.

$$HCy + NH_4HS = 2H + NH_4SCy.$$

White ammonium sulpho-cyanide is formed.

Now touch this with ferric chloride, Fe_2Cl_6, and it turns to blood-red sulpho-cyanide of iron.

$$6NH_4SCy + Fe_2Cl_6 = 6NH_4Cl + Fe_2(SCy)_6.$$

ANTIDOTES.—Affusions of cold water, ammonia, chlorine and a mixture of ferrous sulphate, ferric chloride and magnesia.

PHOSPHORUS, P.

41. Boil a match head in water with a few drops of some acid, and in a dark closet note the luminous appearance.

42. Observe that it has an odor like garlic.

43. Clasp over the mouth of the tube, filter paper moist with silver nitrate. The phosphorus vapor will cause a dark stain of phosphide of silver.

ANTIDOTES.—*Copper Sulphate*, crude turpentine, magnesia in milk.

CHLOROFORM, $CHCl_3$. *Trichlormethane.*

44. A strip of paper wet with chloroform and burned in the Bunsen flame imparts a green tint and yields acid fumes which redden moist blue litmus paper. If the glass rod wet with ammonia is held above the flame, white clouds of ammonium chloride show that HCl is a product of combustion.

45. To an alcoholic solution of potassium hydrate add a few drops of aniline, $C_6H_5.NH_2$, and one of chloroform.

Immediately, or perhaps after gentle heat, a penetrating, disagreeable and peculiar odor is emitted.

$$CHCl_3 + 3KHO + C_6H_5.NH_2 = C_6H_5.NC + 3KCl + 3H_2O.$$

The offensive benzo-isonitril and potassium chloride and water are formed.

ANTIDOTES.—Treat symptoms by lowering the head, drawing out the tongue, cold affusion, artificial respiration, electricity.

CHLORAL HYDRATE, C_2HCl_3O. *Trichloraldehyde.*

46. Dissolve a small piece of chloral hydrate in water, add potassium hydrate and boil.

$$C_2HCl_3O + KHO = CHCl_3 + KCHO_2.$$

Chloroform and potassium formate are formed.

The chloroform vapor may be recognized by its odor or recovered by distillation, and the tests for chloroform applied to the distillate.

47. Apply the aniline test, § 45, to a portion of the mixture in the test tube, and benzo-isonitril may be detected by its offensive odor.

48. Remove the chloroform from the above mixture, § 46, by boiling, then add ammonia and a crystal of silver

nitrate. The silver is reduced with formation of a metallic mirror on the tnbe.

ANTIDOTES.—Wash out the stomach with tea and coffee and treat symptoms with cold affusions, sinapisms to epigastrium, artificial respiration and electricity.

ALCOHOL, $C_2H_5.HO$.

49. To solution of potassium bichromate, $K_2Cr_2O_7$, add a few drops of acid sulphuric, and dilute with water. If alcohol be now added and the admixture gently heated, it will turn green and aldehyde vapor will be recognized by its odor.

$$3C_2H_5OH + K_2Cr_2O_7 + 4H_2SO_4 = 3C_2H_4O_6 + K_2SO_4.Cr_2(SO_4)_3 + 7H_2O.$$

Aldehyde and green potassio-chromic alum are formed.

50. Dissolve a crystal of iodine in some alcohol. Add potassium hydrate until the brown color disappears, and after gentle heat a yellow precipitate of iodoform falls.

$$C_2H_5OH + 6KHO + I_8 = CHI_3 + CHO_2K + 5KI + 5H_2O.$$

Iodoform, potassium formate, potassium iodide and water are formed.

METALLIC POISONS.

ARSENIC, As. ARSENIC TRIOXIDE, As_2O_3. ARSENIOUS ACID, H_3AsO_3.

51. Put into a reduction tube a small quantity of As_2O_3 mixed with some dried flux (made of dry sodium carb. 3 parts to potass. cyanide 1 part). Heat the mixture and observe the formation of a dark mirror ring of As on the tube (Fig. 25). Break off the closed end of the tube and heat the ring. A current of air plays through the tube and oxidizes the As to As_2O_3, which is

deposited in the cool part of the tube as a white ring of minute octahedral crystals (Figs. 26 and 27).

52. To a solution of As_2O_3 add a few drops of HCl and pass through it hydrogen sulphide, H_2S; a lemon-

FIG. 25.

FIG. 26.

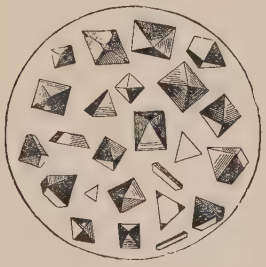


FIG. 27.

yellow precipitate falls, soluble in ammonia, and in NH_4HS, but not in HCl.

$$2H_3AsO_3 + 3H_2S = As_2S_3 + 6H_2O.$$

Arsenic trisulphide and water are formed.

53. To a neutral, alkaline or feebly acid solution of As_2O_3 add silver ammonio-nitrate; it throws down a canary-colored precipitate of silver arsenite, Ag_3AsO_3.

54. To a solution of As_2O_3 add copper-ammonio-sulphate; it throws down a green precipitate of copper arsenite, $CuHAsO_3$.

55. *Fleitmann's Test.*—Into a test tube put some strong potassium hydrate, and a few pieces of pure zinc; clasp

over the mouth of the tube paper wet with silver nitrate, and boil. If the paper is not stained no arsenic is present in the materials used.

$$Zn + 2KHO = H_2 + K_2ZnO_2.$$

Hydrogen and potassium zincate are formed.

Now add a few drops of arsenical solution to the same mixture and boil as before; a black stain appears on the paper.

$$AsH_3 + 6AgNO_3 + 3H_2O = 6Ag + H_3AsO_3 + 6HNO_3.$$

Black metallic silver, arsenious acid and nitric acid are formed.

56. *Reinsch's Test.*—Into a solution of As_2O_3 in a test tube put a few drops of HCl and a clean slip of copper; then boil for a few minutes. When the slip has a steel gray coat of As, remove it; dry with blotting paper, and heat in an open glass tube. Note the white ring of As_2O_3 crystals.

57. *Marsh's Test.*—Into a hydrogen-generating apparatus put some pure zinc and add dilute sulphuric acid (H_2SO_4 part one to H_2O parts four). *Wait* fifteen minutes, until the explosive mixture of air and hydrogen has escaped, then light the H gas at the jet and hold in the flame a piece of cold porcelain. If no stain is made then arsenic and antimony are absent from the materials. Now add solution of arsenious acid and try the flame again with the

FIG. 28.

porcelain. A brownish black metallic mirror of As appears.

$$3Zn + 3H_2SO_4 + H_3AsO_3 = 3ZnSO_4 + 3H_2O + AsH_3.$$

Zinc sulphate, water and hydrogen arsenide are formed in the generator.

When the AsH_3 is burned it is decomposed, forming H_2O + As. The arsenic, when deposited on the cold porcelain is recognized by being quickly dissolved when treated with liq. sodæ chloratæ or a solution of calx chlorata. If treated with nitric acid and gently evaporated, it is oxidized to arsenic acid as a whitish deposit. The arsenic acid touched with a drop of strong solution of $AgNO_3$ gives red silver arseniate. The original As deposit on porcelain will dissolve slowly in ammonium sulphide, leaving a yellow residue on evaporation. This residue is soluble in NH_4HO.

58. Use a fresh hydrogen apparatus with solution of an antimony salt, say tartar emetic, repeating the same procedure. On the porcelain a sooty stain appears, which is not removed on adding a drop of liq. sodæ chloratæ. It is soluble in nitric acid but does not afterward turn red with silver nitrate. It is freely soluble in ammonium sulphide with an orange-red residue insoluble in NH_4HO.

59. Make the *antidote, ferric hydrate,* by adding to tinctura ferri chloridi some calcined magnesia or aqua ammonia in excess and shake well together. 3 fluid-ounces of tinct. ferri chlor. yields antidote for 10 grains of arsenious acid.

$$2(Fe_26HO) + As_2O_3 = Fe_32AsO_4 + 5H_2O + Fe2HO.$$

ANTIDOTES TO ARSENIC.—Milk, eggs, hydrate magnesia, dialysed iron, *moist ferric hydrate,* ferri oxidum hydratum cum magnesia.

ANTIMONY, Sb. TARTAR EMETIC, $K(SbO)\ C_4H_4O_6$.

60. Pass hydrogen sulphide into a solution of tartar emetic, acidified with dilute HCl or tartaric acid. An orange-red precipitate of antimony sulphide forms. Divide this precipitate in three portions; one is insoluble in aq. ammonia, one is soluble in ammonium sulphide and the third soluble in hydrochloric acid. Arsenic sulphide is insoluble in HCl.

61. Repeat Reinsch's test, § 56, using solution of tartar emetic instead of arsenic. The deposit on copper is bluish, and when heated in an open glass tube yields a white *amorphous* sublimate. If this sublimate is dissolved in HCl and evaporated the residue turns orange with H_2S.

62. Repeat Marsh's test, § 57, using solution of tartar emetic instead of arsenic. The stain on porcelain is sooty, barely soluble in NaClO but soluble in NH_4HS, leaving an orange-red residue.

63. Put a drop of solution of tartar emetic on platinum foil, add a drop of HCl, and touch the platinum through the drop with a slip of zinc. A black spot of antimony appears which turns orange-yellow with ammonium sulphide.

ANTIDOTES TO ANTIMONY.—*Acid tannic*, tea, vegetable astringents, dialysed iron, moist ferric hydrate, stomach pump.

COPPER, Cu.

64. Treat acidulated solution of copper sulphate, $CuSO_4$, with hydrogen sulphide, and a brown-black precipitate falls.

65. Dip into solution of $CuSO_4$ a bright steel needle; it soon has a red coat of metallic copper.

66. Treat a weak solution of $CuSO_4$ with potassium ferrocyanide; a brown precipitate falls.

67. To solution of $CuSO_4$ add a drop of ammonia; a bluish precipitate falls, but on addition of more ammonia this clears into a deep blue solution.

$$CuSO_4 + 2NH_4HO = Cu2NH_3.SO_4 + 2H_2O,$$

Blue ammonio-sulphate of copper and water are formed.

ANTIDOTES.—Warm milk and raw eggs.

MERCURY, Hg. CORROSIVE SUBLIMATE, $HgCl_2$.

68. Treat calomel, Hg_2Cl_2, with lime water; it turns to black oxide, Hg_2O.

$$Hg_2Cl_2 + Ca2HO = Hg_2O + CaCl_2 + H_2O.$$

Black mercurous oxide, calcium chloride and water are formed.

69. Treat corrosive sublimate, $HgCl_2$, with lime water; it turns to yellow oxide, HgO.

70. Perform Reinsch's test, § 56, with corrosive sublimate solution without boiling; the copper takes a gray coat of metal, Hg.

Dry the copper and heat it in an open tube; a sublimate of minute gray globules forms. Heat this sublimate with iodine; it forms red mercuric iodide, HgI_2.

Galvanic Test.—On a gold ring or coin put a drop of solution of corrosive sublimate; with a steel needle or knife touch the gold through the drop, and a silver-like spot of metal, Hg, appears. This stain is removable by gentle heat over a flame.

ANTIDOTES.—Raw eggs, flour in milk, emetics.

Lead, Pb.

71. Put a crystal of lead acetate on a piece of charcoal and heat with the tip of a blowpipe flame. A globule of metallic lead soon forms and a yellow margin of lead oxide surrounds it on the charcoal.

72. To a solution of a lead salt add hydrogen sulphide; a black precipitate of lead sulphide, PbS, falls; insoluble in KHO and NH_4HS, but soluble in boiling dilute HNO_3.

73. To another portion of lead solution add acid sulphuric; a white precipitate of lead sulphate, $PbSO_4$, falls.

$$PbCO_3 + MgSO_4 = PbSO_4 + MgCO_3.$$

74. To another portion add potassium iodide; a yellow precipitate of lead iodide, PbI_2, falls.

75. To another portion add K_2CrO_4; a yellow precipitate of lead chromate appears.

Antidotes.—Magnesia sulphate and other soluble sulphates with milk and opium for colic, potassium iodide and iron for chronic poisoning.

Silver, Ag.

76. To a solution of silver nitrate add H_2S; it precipitates black silver sulphide Ag_2S, insoluble in dilute acids, alkalies, alkaline sulphides, but soluble in boiling HNO_3.

77. To another portion cautiously add NH_4HO; it gives a brown precipitate of Ag_2O, which readily dissolves in excess of NH_4HO.

78. Treat fresh portions successively with HCl = white curds of AgCl, soluble in NH_4HO. KI = yellow ppt. of AgI, insoluble in NH_4HO. H_2SO_4, yields no precipitate whatever.

Antidotes.—Common salt, raw eggs and milk.

Zinc, Zn.

79. To solution of a zinc salt add ammonium hydrate. A white precipitate of zinc hydrate, $Zn(OH)_2$ falls, soluble in excess of the ammonium hydrate.

80. To another portion add first ammonium chloride and then ammonium hydrate. There is no precipitate. Now add hydrogen sulphide. White zinc sulphide is thrown down.

81. To a neutral or alkaline solution of a zinc salt add ammonium sulphide. A white precipitate, ZnS, is thrown down.

Antidotes.—Raw eggs, milk, tea, vegetable astringents.

Barium, Ba.

82. From solution of a barium salt, potassium hydrate will throw out white barium hydrate, $Ba(OH)_2$. Ammonium hydrate has no such effect when added to another portion.

83. To the clear solution with ammonia add sodium phosphate, Na_2HPO_4; it forms a white precipitate of barium phosphate, $BaHPO_4$.

84. To a solution of barium salt add acid sulphuric or any soluble sulphate. A white precipitate, $BaSO_4$, falls.

Antidotes.—Magnesium sulphate or any soluble sulphate, eggs and milk.

85. ANALYSIS OF A NEUTRAL OR SLIGHTLY ACID SOLUTION OF ORDINARY SALTS OF ONE OF THE COMMON METALS. (*Attfield.*)

Add hydrochloric acid.

Precipitate Hg(ous) Pb Ag. Collect, wash, and add NH_4HO. Hg ppt., blackened. Pb ppt., still white. Ag ppt., dissolved. Sb and Bi may also be precipitated by HCl, but are dissolved on adding more HCl.

If HCl gave no precipitate the metal is still in the liquid; pass H_2S through the solution.

Precipitate Cd Cu Hg(ic) Pb Bi As Sb Sn Au Pt. Collect, wash, add NH_4HS.

Insoluble		Soluble.	
Cd, yellow.		As(ous & ic)	yellow.
Cu	black.	Sn(ic)	yellow.
Hg(ic)	black.	Sb, orange.	
Pb	black.	Sn(ous)	black.
Bi	black.	Au	black.
		Pt	black.

Apply special tests for each to the original solution. For these, see the previous pages.

If H_2S gave no precipitate add NH_4Cl, NH_4HO and NH_4HS.

Precipitate Zn Mn Co Ni Al Fe Cr.

Zn, Al } white.
Cr, green.
Mn, skin-tint.
Ni, Co, Fe } black.

Test specially for each in original solution. See previous pages.

If NH_4HS gave no precipitate, add $(NH_4)_2CO_3$.

Precipitate Ba Sr Ca. Collect, wash, dissolve in $HC_2H_3O_2$, add K_2CrO_4.

Ppt. Ba.	Sol. Sr Ca Add dil. H_2SO_4.
	Ppt. Sol. Sr.—Ca.

If $(NH_4)_2CO_3$ gave no precipitate, add $(NH_4)_2HAsO_4$.

Ppt. Mg.	If no precipitate, test original solution in flame on loop of Pt wire. L, crimson. Na, yellow. K, violet. If neither, test orig. sol. for NH_4.

ALKALOIDS.

STRYCHNINE, $C_{21}H_{22}N_2O_2$.

86. Note the intensely bitter taste of one drop of the solution.

87. On a white dish put a crystal of strychnine and add to it a drop of acid sulphuric. It dissolves without change of color. Near by upon the same dish put a crystal of potassium bichromate and let the acid solution flow over it. At the line of contact a transient play of colors will be seen in the following order: blue, violet, red and yellow.

88. Nitric acid makes with it a colorless solution. If brucine be present as an adulteration a red color is produced.

89. Insert a few drops of strychnine solution under the skin of a frog's back. Within a few minutes it will be convulsed.

ANTIDOTES.—Emetics with draughts of tea or other vegetable astringents, chloroform by inhalation, chloral and potassium bromide by enema.

MORPHINE, $C_{17}H_{19}NO_3$.

90. On a white dish put a crystal of morphine and touch it with a drop of nitric acid; it turns at first orange red and fades to yellow.

91. To a neutral solution or a crystal of morphine add pure neutral ferric chloride, Fe_2Cl_6; a deep blue color appears.

92. To morphine solution add iodic acid in solution; a yellow color of reduced iodine appears. If the yellow

liquid is shaken with chloroform, the iodine separates in a violet-colored layer.

ANTIDOTES.—Emetics, washing out the stomach with strong tea, electricity, flogging, artificial respiration, brandy.

OPIUM. MECONIC ACID.

93. To a portion of solution of meconic acid or a decolorized infusion or tincture of opium add ferric chloride, Fe_2Cl_6 (pure and neutral); a brown-red color appears. This color persists even if treated with mercury bichloride, $HgCl_2$, or after boiling with dilute HCl.

ATROPINE, $C_{17}H_{23}NO_3$.

94. A small portion of atropine, if heated with strong acid sulphuric, does not change color, but gives off an odor of roses.

95. Into a test tube put a few drops of acid sulphuric and a crystal of potassium bichromate, and heat them. If a fraction of a grain of atropine is now dropped in, a green color and an aromatic odor may be perceived.

96. ANALYTICAL TABLE FOR COMMON ALKALOIDS.—(*Senier's.*)

PART I.

MORPHINE. BRUCINE.		SALICIN. STRYCHNINE.	
To a small quantity on a white plate add strong nitric acid.		If no Morphine or Brucine, moisten a small quantity on a white plate with strong sulphuric acid.	
An Orange Color, decolorized by Sn Cl_2, $Na_2S_2O_3$, or NaHS = *Morphine.** Confirm by—Fe_2Cl_6 (neut.), which gives with morphine or its salts a blue color. HIO_3 is decomposed by morphine or its compounds, with liberation of iodine, which may be recognized by starch.	*A Red Color.* To a few grains in a test tube add a drop or two of HNO_3; warm to boiling and dilute with water; then add a few drops of stannous chloride. A violet color = *Brucine.*	*A deep red color = Salicin.* Confirm by boiling the substance with water to which has been added a few drops of dilute H_2SO_4. Then make solution alkaline and examine for glucose.	*No Color, or only a slight color.* Draw a moistened crystal of $K_2Cr_2O_7$ across the acid film when a transient play of colors, violet to red = *Strychnine.*

* Strychnine of commerce often gives an orange or red color, due to contamination with brucine.

ANALYTICAL TABLE FOR COMMON ALKALOIDS.

PART II.

QUININE.	CINCHONINE.
QUINIDINE.	CINCHONIDINE.

If Morphine, Brucine, Salicin, and Strychnine are absent, make a saturated solution in hot water and cool down to about 60° F., removing the crystals which have deposited by filtration. If the substance is an alkaloid or an acid salt, it must be neutralized. To one volume of this solution in a test tube add exactly half a volume of ether and then ammonia solution in slight excess. Agitate and set aside for a quarter of an hour.

Precipitate entirely soluble in the ether.	*A crystalline layer between the ethereal and aqueous solutions.*	*Precipitate insoluble in ether = Cinchonine.*
To a portion of the saturated aqueous solution add rather more than an equal bulk of ammonia solution (sp. gr. 0.96) and agitate. The precipitate dissolves completely = *Ordinary Sulphate of Quinine.* If precipitate does not dissolve completely, add to another portion of the aqueous solution $(NH_4)_2C_2O_4$ and agitate (no precipitate at once with ordinary sulphate of quinine). A bulky crystalline precipitate = *Quinine.*	To a portion of the aqueous solution add $Na_2C_4H_4O_6$ and agitate. A bulky crystalline precipitate = *Cinchonidine.* If no precipitate or only a trace, add to another portion of the aqueous solution KI. A heavy white precipitate = *Quinidine.* If the precipitate be semi-liquid, it contains cinchonidine or other alkaloids, the hydriodates of which are soluble in weak spirit.	Confirm by other reactions (see Cinchonine).

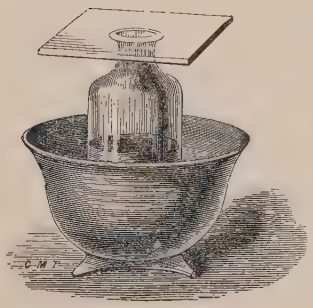
PRELIMINARY EXAMINATION OF VOMITED OR OTHER SUSPECTED MATTERS.

1. Note stains on the clothing or about the lips and mouth.

2. Put in a white dish and examine for odor, seeds, wing cases of cantharides, crystals or powders.

3. Take a small measured portion, say one-sixth or one-third; put in a clean jar and stand in a bowl of warm water.

Fig. 29.



4. *Test for volatile poisons.*

HCy by odor and by action of vapor on reagents applied to the under side of a glass cover to the jar. § 38, 39, 40.

Phosphorus by odor and luminous vapor evolved in a dark room, by boiling a portion in a test tube. § 41, 42, 43.

Carbolic acid by odor. § 33, 34, 35, 40.

5. Test for *corrosive acids* by litmus paper, by examining stains on clothing, and by analyzing a sample of suspected substance.

6. Test for *corrosive alkalies* by litmus paper or direct testing of suspected sample.

7. Test for *metallic irritants.*

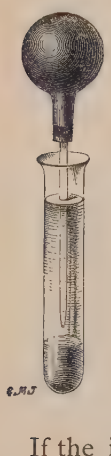
Apply Reinsch's test, § 56, for As, Sb, Hg; if there is a metal deposit on the copper, then make a subli-

mate in an open glass tube. Examine sublimate with lens, wash out with HCl, evaporate, and test residue with H_2S.

If there is no deposit on the copper acidulate a portion with HCl and add H_2S. If it forms a black precipitate test for Cu and Pb. § 66, 74, 75.

8. If the patient has narcotism or convulsions, apply Stas' method for *morphine* or *strychnine* by making a portion of the fluid vomit alkaline with sodium carbonate, which sets the alkaloid free. The alkaloid is then separated from the liquid which holds it suspended by agitating it with four volumes of ether or amylic alcohol. By letting the mixture stand, the ether floating will hold the alkaloid in separate solution, and may then be removed by a pipette. (Fig. 30.)

FIG. 30.



If the ether is allowed to evaporate on a saucer or watch glass the residue may be examined for strychnine, § 86–89, and morphine, § 90–92.

If the indications point to morphine, then agitate a fresh portion of the original vomited fluid with amylic alcohol, as it is a better solvent for that alkaloid, separate, evaporate and test, § 90–92.

Note.—The methods indicated above are "rough and ready," and are such as might be applied quickly to vomited matter by a practitioner between his visits. They may serve to clear up a diagnosis before it is too late to help the patient. They would not answer for a juridical trial. The methods applied by expert analysts are far more searching, and require much more apparatus, time and special knowledge.

Before the analyst begins testing for the metallic irritants he first gets

rid of all organic matter by some such process as that of Fresenius. The finely divided substance is boiled with pure hydrochloric acid, while potassium chlorate is added from time to time until the solids are reduced to a straw-yellow fluid. This fluid is then treated with excess of sodium bisulphite and saturated with hydrogen sulphide until the metals are thrown down as sulphides. These sulphides are then treated with various tests for the metals.

STUDY OF NORMAL MILK

AND

MILK EXAMINATION.

COMPOSITION OF NORMAL MILK.

Milk is a sweet, opaque, bluish-white fluid with a peculiar odor (holding in solution casein, albumin, sugar and mineral salts). Its turbidity is due to the minute fat or butter globules which are suspended in it.

Percentage Composition of Normal Milk.	Cow.	Human.
Water,	85.70	88.90
Solids, as tabulated below,	14.30	11.10
Casein,	4.82	3.90
Albumin,	0.58	...
Butter,	4.30	2.65
Milk Sugar,	4.00	4.35
Salts,	0.50	0.20

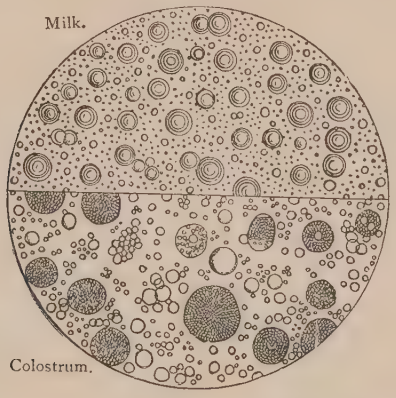
The composition is somewhat variable, the proportion of casein rising when the breasts are emptied frequently, and the last portions obtained containing more butter.

Microscopically, the milk is found to be composed of minute brilliant *oil globules* in thin envelopes of casein suspended in the clear milk plasma. Immediately after delivery of the young, the milk is relatively poor in casein but rich in fatty matter, which exists to a considerable extent in the form of colostrum masses. (Fig. 31.)

1. *Reaction.*—With red litmus paper test human milk; it turns blue, showing alkalinity. Cow's milk is usually alkaline when fresh, though sometimes acid, and occasionally a sample will be found that is amphoteric, *i. e.*, reddens blue litmus paper and turns blue litmus paper red.

2. *Spontaneous Change.*—Let milk stand for several days in a warm place; it coagulates and "*sours*," *i. e.*, turns acid.

FIG. 31.



This change is due to the conversion of the milk sugar into lactic acid by a minute organism. The casein is normally held in solution by alkaline phosphates. Acids change the reaction and thus cause the casein to be precipitated.

3. To half a test tube of diluted milk add a drop or two of hydrochloric or acetic acid and gently warm it; an abundant precipitate falls. This precipitate is the curdled casein, carrying with it most of the fat.

4. *Milk Sugar and Salts.*—Having filtered the whey from the acid curd, § 3, test the whey with Fehling's

solution. The milk sugar reduces the cupric oxide. To another portion add magnesia mixture; the phosphates are precipitated. To another portion add silver nitrate; the chlorides are precipitated, insoluble in nitric acid.

5. *Butter.*—To a test tube one-third full of milk add half its volume of potassium hydrate and half of ether; shake the mixture and stand in a warm place. The milk clears up, and the butter dissolved in the ether floats at the top. On separating the ethereal layer and evaporating it a residue of butter is left.

In cow's milk ether will not dissolve the fat globules unless they are liberated by dissolving their envelopes with acetic acid or caustic potash or soda. With human milk it suffices to agitate with ether alone. In ordinary churning the envelopes of casein are ruptured mechanically, and the fat globules cohere in large masses of butter.

6. The *Boiling Scum.*—Put some milk in a capsule and boil it for some time. A scum of coagulated casein or albumin forms on the surface, but below this pellicle no curd is formed.

7. *Pepsin Curd.*—Into a test tube about one-third full of milk, put a few drops of neutral essence of pepsin (Fairchild's). Mix gently, warm to the temperature of the body, and keep at 104° F. (40° C.). A solid *curd* forms in 10 or 12 minutes, so that the tube can be inverted without losing the milk. In a short time a *whey* separates from the clot.

8. *Junket, or Curds and Whey.*—To make this delicacy for invalids, take one-half pint of fresh milk heated as hot as can be agreeably borne by the mouth, add one teaspoonful of rennet, or essence of pepsin (Fairchild), and stir just enough to mix. Let it stand till firmly curded; may be served plain or with sugar and grated nutmeg.

An egg beaten to a froth and sweetened with two teaspoonfuls of sugar

may previously be added to the half pint of milk, forming a highly nutritious and smooth jelly. The essence will curdle milk with egg as readily as plain milk.

9. *Whey.*—Curdle warm milk with the pepsin essence as above directed; then beat up with a fork until the curd is finely divided, now strain and the whey is ready for use.

Whey is useful in diet for the sick. It is frequently resorted to as a food for infants to tide over periods of indigestion.

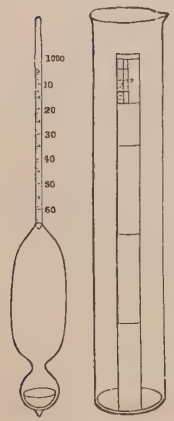
10. To the curd obtained, § 7, add a few drops of dilute hydrochloric acid, so as to make with the pepsin an artificial gastric juice, and set aside at 104° F. for two or three hours. The curd is digested and gradually dissolves to make a yellowish fluid with the peculiar odor and bitter taste of "peptonized milk."

11. *Rennet Curd.*—To a small quantity of fresh milk in a test tube add a few drops of commercial extract of rennet, warm to 104° F. and set aside. In a few minutes a solid coagulum forms, and later on a sweet whey separates.

If the milk be previously boiled, or if the rennet be boiled, the ferment will not work.

It acts best at a temperature that can be borne in the mouth, *i. e.*, about 104° F.

FIG. 32.



Hydrometer (*Starr*). Creamometer (*Starr*).

12. *Specific Gravity.*—Shake well a specimen of fresh or "whole milk," and pour into a cylindrical glass vessel until two-thirds full. Slowly immerse a hydrometer; it will record at the surface somewhere between 1029 and 1034 (Fig. 32).

13. The specific gravity of dairy milk, the product of a number of cows, should never fall below 1029. When lower than this it is usually due to

adulteration with water, but very rarely the low density is due to excess of cream in very rich milk.

14. Let the milk stand for 24 hours, "skim" and remove the "cream" which has risen to the surface. Now take the specific gravity: it will range between 1032 and 1040.

The fat globules of the cream in "whole milk" lower the gravity, because fat is lighter than water. "Skim milk" is heavier than fresh or "whole milk," but this difference may be removed by adding water, which is also lighter than milk.

15. *Correction for Temperature.*—As cold milk is heavier than warm milk—*i. e.*, has a higher specific gravity—a correction for temperature is necessary. For ordinary hydrometers, adjusted at 60 F., an approximatively correct specific gravity is obtained by subtracting 1 from the hydrometer reading for each 10° F. *below* 60° F., and by adding 1 to the reading for each 10° F. above 60° F. For variations of less than 10° F., the proportionate fraction of 1 must be added or subtracted, as the case may be.

MILK TESTING.

(*a*) *Take the specific gravity with a hydrometer and correct for temperature.* (Fig. 32.)

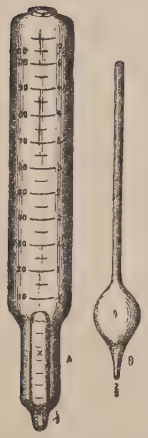
Any milk that stands above 1033 (33 of the scale) is almost sure to be skimmed, while that falling below 29 of the scale is nearly always watered. The only exception to this rule is in the case of a very rich milk containing an excess of cream. This excess would be apparent to the unaided eye by the consistency and the "creamy" appearance. There is a trick known to the milk trade by which the indications yielded by the specific gravity may prove fallacious. Some of the cream is skimmed off of fresh milk with a specific gravity of about 1030, leaving a milk of higher specific gravity, about 1035. By adding water in proper amounts the specific gravity may be lowered again to that of the original whole milk. The trained eye may detect a change in color and consistency, but we cannot depend on the *creamometer* (a graduated cylinder by which we measure the layer of cream that rises in a certain time). A milk that has been watered will in a few hours separate a thicker layer of cream than would the same amount of the unwatered. The separation takes place with greater rapidity in the diluted

than in the whole milk. The *lactometer* of the N. Y. Board of Health is a hydrometer which has a scale on which 100° stands for a specific gravity of 1029 (the minimum density of pure milk), while 0° stands for the specific gravity of water, and 120° for 1034, the maximum range of pure milk. On this instrument 1° is read as one per cent. of milk in the sample.

(*b*) *Note the color and consistency.*

A rich milk looks like cream, and is thicker than a skimmed milk. If the color is bluish or less creamy, and the fluid less opaque, while the specific gravity is less than 1029 or under 100° of the New York lactometer, then it is safe to assume that the milk has been watered.

FIG. 33.



Feser's Lactoscope (*Queen.*)

A pure, rich milk is perceptibly more opaque than a skimmed or watered sample. The opacity or white color of the milk is due to the suspended fat globules, and is proportionate to the number of them. By measuring this opacity an approximate estimate can be made of the percentage of fat. For making this estimate roughly, Heeren's pioscop may be used, but *Feser's lactoscope* (Fig. 33), is very convenient and more accurate. In the axis of a cylindrical, clear glass vessel, and at its lower part, A, is a smaller cylinder of white glass, marked with a few black lines. In testing with this instrument 4 c.c. of milk are introduced with the graduated pipette, entirely concealing the black lines. Pure water is gradually added, while shaking, until the milk clears up sufficiently to make the black lines visible. By the graduation on the vessel the surface level of diluted milk can be read as percentage of fat in the original sample. The microscope having determined the absence of chalk, starch or other suspended adulterants, a sample showing 3 per cent. and over is judged pure. Some rich Jersey milk shows 6 per cent. Any one experienced in its use will be accurate to within ¼ of 1 per cent. Having obtained the specific gravity by the lactometer, and the percentage of fat by the lactoscope, experiment shows that the proportion of *solids not fat* can be calculated by the formula :—

$$\text{Solids not fat} = \frac{S - A}{0.00375}.$$

S standing for the hydrometer reading (as 30 for 1030), and A for the remainder obtained by multiplying the percentage of fat shown on the lacto-

scope by 0.001 and subtracting the product from 1.0000. A good milk should indicate from 9 to 12 per cent.

(*c*) *Measure the amount of cream.* (Fig. 32.)

For this purpose a creamometer or a 100 c.c. glass cylinder graduate may be used. Having mixed the milk thoroughly, a sample is poured into the vessel up to the highest mark. After standing in a cool place for twenty-four hours the depth of cream layer thrown up is read off. It ought to show from 10 to 20 volumes in the hundred. The average sample would be 12 per cent. If the cream forms 20 per cent. of the column, the sample would probably also show a low specific gravity. The accuracy of this test is affected by the length of time since milking, by the amount of previous agitation of the milk, by the fact that dilution causes a more rapid separation of the cream, by the temperature and other variable conditions. It may serve a useful purpose when taken in consideration with other observations.

Less than 10 per cent. of cream in a milk of specific gravity above 1033 denotes skimming.

Less than 20 per cent. of cream, if joined to a specific gravity less than 1029, indicates watering.

DETERMINATION OF WATER, SOLIDS, ASH AND FAT.

The chemical standards of milk for the State of New York are that it shall contain not more than 88 per cent. of water, not less than 12 per cent. of milk solids, nor less than 3 per cent. of fat.

Water.—Into a tared dish, preferably of platinum, five grammes of milk are weighed. The dish is then exposed to the heat of a water bath for three hours. As evaporation is nearly done, it is now put into a water oven and at intervals weighed until it ceases to lose weight. This constant weight, less the weight of the capsule, gives the total solids. The difference between the 5 grammes and the constant weight of the dry solids represents the *water*. By carefully incinerating the

solids to a grayish-white color the *ash* or inorganic salts are determined. In pure milk the amount ranges from 0.70 to 0.80 per cent. A watered milk will show a reduced amount both of solids and of ash.

Fat.—To determine the butter by the gravimetric method, 10 grammes of milk are weighed into a tared dish containing a weighed amount of dry sand. The milk is evaporated on a water bath and last on a water oven, with constant stirring. The residue is washed a number of times with warm ether or petroleum naphtha of 70° Baumè and the washings passed through a small filter. The filtrates are all received in a tared beaker and carefully evaporated to a constant weight. The residue is *fat.* This subtracted from the amount of total solids gives the *solids not fat.*

7.H.24.
The urine, the common poisons, 1891
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